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THE EFFECT OF LEVELS OF SODIUM SALTS IN THE FEED AND DRINKING WATER ON THE OCCURRENCE OF ASCITES AND EDEMA IN TURKEY POULTS

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ABSTRACT

An experiment was conducted to study the effects of levels of sodium salts in the feed and drinking water on the occurrence of ascites and edema in turkey poults. Poults were fed rations containing four levels of added sodium chloride: 0, 0.5, 1.0 and 2 per cent in combination with six levels of a 3:2:1 mixture of Na_2SO_4; NaCl and $NaHCO_3$ added to the drinking water; 0, 750, 1500, 3000, 4500 and 6000 parts per million.

Rate of mortality and incidence of ascites and edema were influenced by the amount of salt added to the feed as well as by the concentration of salts in the drinking water. With higher levels of salt in the feed lower levels of salts in the water were toxic, while with low levels of salt in the feed poults were able to tolerate higher levels of salts in the drinking water.

Mortality and incidence of ascites and edema increased sharply at 3 days of age, reached a peak at 6 days of age and declined and stopped after 12 days on treatment.

INTRODUCTION

The occurrence of an edematous condition in poults, sometimes referred to as "water-belly", may cause heavy losses to turkey producers. While often there does not appear to be a clear-cut explanation for its appearance in the field, frequently the disorder has been ascribed to excessive levels of sodium salts in the feed or drinking water. Experimentally, the condition has been associated with a high sodium intake (2, 3, 6).

While excessive intake of salt from either the feed or water supply may prove toxic, experiments with chicks have shown that there is a lesser tolerance for salt in the drinking water. Mitchell, Card and Carman (5) observed that salt given in solution twice daily was more toxic than an equal amount given in the feed. Selye (7) reported that the fowl is comparatively sensitive to small doses of sodium chloride in the drinking water. A 0.9 per cent solution was found to be extremely toxic, resulting in water retention and marked renal changes. Other work relating to the toxicity of salt for chickens has been reviewed by Barlow *et al.* (1) and Kare and Biely (4).

The low tolerance of poults for salts in the drinking water has focused attention on possible toxic effects which might arise from levels and type of minerals common in well waters. One of the outstanding features of water

supplies in Alberta is the variability which exists. Some are low in minerals (200-300 p.p.m.) while others may contain levels as high as 20,000 parts per million. A large percentage of wells have water containing from 1000 to 2000 p.p.m. of total solids. In some well waters one or two salts predominate; in others complex mixtures of salts are found.

The types of water encountered may be classified on the basis of the salts present which in turn appear to be related to the geological structure in which the water is found*. In shallow wells (up to 50 feet in depth and falling in the glacial drift) sodium, magnesium and calcium bicarbonates and sodium sulphate are the principal salts. In deeper wells (50-100 feet in depth) which reach bedrock formations, the calcium and magnesium concentrations are lowered owing to ion exchange in which calcium and magnesium are replaced by sodium. Thus the predominant salts are sodium bicarbonate and sodium sulphate. In still deeper wells, an increase in sodium occurs until brackish waters are reached. The salts found are principally sodium bicarbonate, sodium sulphate and sodium chloride. When a depth of about 300 feet is reached, sodium chloride tends to replace sodium sulphate and below 400 feet sodium chloride is the principal salt found. In general, in recharge areas, water tends to be high in sulphates while in discharge areas, such as bedrock channels (probably representing pre-glacial rivers) and in areas close to river valleys, chlorides predominate. Total solids present in well waters are usually higher in discharge areas and lower in recharge areas.

Since the levels of salts in feed and water supplies may be quite variable the experiment reported herein was conducted to study the relationships between level of sodium chloride added to a ration, the level of salts in the drinking water and the occurrence of ascites and edema in turkey poults.

MATERIALS AND METHODS

Four hundred and eighty day-old Broad Breasted Bronze poults, of mixed sexes, were divided into 24 comparable lots of 20 birds each. The poults were fed rations containing four levels of added sodium chloride, 0, 0.5, 1 and 2 per cent, in combination with six levels of salts in the drinking water. The levels of salts added to the water supply were 0, 750, 1500, 3000, 4500 and 6000 p.p.m. The salts were added to Edmonton city water which contains approximately 100 p.p.m. of total solids. The salt mixture used to make the solutions consisted of sodium sulphate, sodium chloride and sodium bicarbonate in the proportions of 3:2:1 by weight. The mixture was selected as representative of the principal sodium salts found in Alberta water supplies.

The poults were raised in electrically heated batteries with raised screen floors. Feed and drinking water were supplied on a free choice basis. The water supply was renewed each morning and replenished when necessary during the day. The basal turkey starter ration used is shown in Table 1. The ration was calculated to contain 26 per cent of protein, 783 calories of productive energy, and the equivalent of 0.46 per cent of common salt.

*LeBreton, E. G.

TABLE 1. — BASAL RATION

Ground corn	20.0
Ground wheat	15.0
Ground oats	5.0
Ground barley	5.0
Wheat bran	5.0
Wheat shorts	6.0
Dehydrated alfalfa meal (17% protein)	5.0
Meat meal (50% protein)	12.5
Fish meal (72% protein)	5.0
Soybean oil meal (44% protein)	18.0
Ground limestone	2.0
Insoluble grit	1.0
Fish oil (2250A, 300D/g.)	0.5
Manganese sulphate (28% manganese) lb./ton	0.5

Vitamins and antibiotic were added at the following levels per pound of ration: Vitamin D_3 750 I.C.U.; riboflavin 1.5 mg.; calcium pantothenate 4.5 mg.; niacin 7.5 mg.; choline chloride 227 mg.; biotin .125 mg.; vitamin B_{12} .003 mg., and procaine penicillin G 2 mg.

TABLE 2. — EFFECT OF TREATMENT ON OCCURRENCE OF MORTALITY, INCIDENCE OF ASCITES AND EDEMA AND AVERAGE WEIGHT

Lot No.[1]	NaCl in ration	Salts[2] in water	Mortality	Ascites and edema	Av. wt. at 14 days
	%	p.p.m.	%	%	gm.
1	0	0	0	0	197
2	0	750	0	0	230
3	0	1500	0	0	239
4	0	3000	0	0	219
5	0	4500	10	0	224
6	0	6000	20	10	231
7	0.5	0	5	5	200
8	0.5	750	5	0	220
9	0.5	1500	5	0	239
10	0.5	3000	5	0	215
11	0.5	4500	5	0	233
12	0.5	6000	25	10	222
13	1.0	0	10	0	211
14	1.0	750	5	0	225
15	1.0	1500	10	5	234
16	1.0	3000	10	10	230
17	1.0	4500	30	20	227
18	1.0	6000	45	40	221
19	2.0	0	5	5	286
20	2.0	750	20	20	231
21	2.0	1500	30	20	169
22	2.0	3000	65	60	265
23	2.0	4500	50	45	232
24	2.0	6000	50	45	253

[1] 20 poults/lot
[2] Salts consisted of Na_2SO_4: NaCl: $NaHCO_3$ in the proportions of 3:2:1 by weight.

Additions of sodium chloride were made at the expense of corn. The poults were weighed and the experiment terminated after 2 weeks of treatment. A record was kept of mortality. An autopsy was conducted on all birds that died and incidence of ascites and edema was noted. Poults that survived were not examined for incidence of the conditions.

The occurrence of ascites and edema was assessed in the following way. Birds which showed an abnormal accumulation of fluid in the abdominal cavity were designated as having ascites while those with accumulation of fluid in the tissues were considered to have edema. No attempt was made to grade the severity of the disorders. Since the conditions occurred together in the poults examined, they are combined in the data reported.

From the data collected the pattern of mortality and the occurrence of ascites and edema during the experimental period was obtained. Daily mortality was obtained on the basis of the number of poults that died each day expressed as a percentage of the total number of poults started; occurrence of ascites and edema in the poults that died was derived and expressed in a similar manner.

RESULTS AND DISCUSSION

An outline of the treatments used and of the results obtained is shown in Table 2. It is apparent that the level of mortality and the incidence of ascites and edema may be influenced either by the level of sodium chloride in the feed or by the addition of sodium-containing compounds to the drinking water.

The average weights of poults receiving the various amounts of salt in the feed and drinking water were not markedly affected by the treatments used. In one group (Lot 21) the average weight of the surviving poults was low; however, higher levels of salt in the drinking water failed to show a similar depression in growth rate.

It is interesting to note that when sodium chloride was added to the ration at levels of 0 or 0.5 per cent practically no mortality from ascites and edema occurred until the concentration of salts in the drinking water reached 6000 p.p.m.

When 1 per cent of sodium chloride was added to the ration (Lots 13-18) the level of salts in the drinking water exerted a more profound effect. Some ascites and edema was noted in the group receiving 1500 p.p.m. of salts in the drinking water (Lot 15). The rate of mortality increased as the amounts of salts in the drinking water was increased with the incidence reaching 40 per cent in the birds receiving 6000 p.p.m. (Lot 18).

When 2 per cent of sodium chloride was added to the ration (Lots 19-24) toxicity was noted at all levels of salts in the drinking water. Even when Edmonton water was given to the birds some ascites and edema occurred (Lot 19). Increasing the concentration of salts in the drinking water to 750 p.p.m. (Lot 20) caused a marked increase in mortality from ascites and edema. The highest mortality noted in this experiment occurred in the group receiving 2 per cent of salt in the ration and 3000 p.p.m. salts in the drinking water (Lot 22) with the level of mortality being somewhat

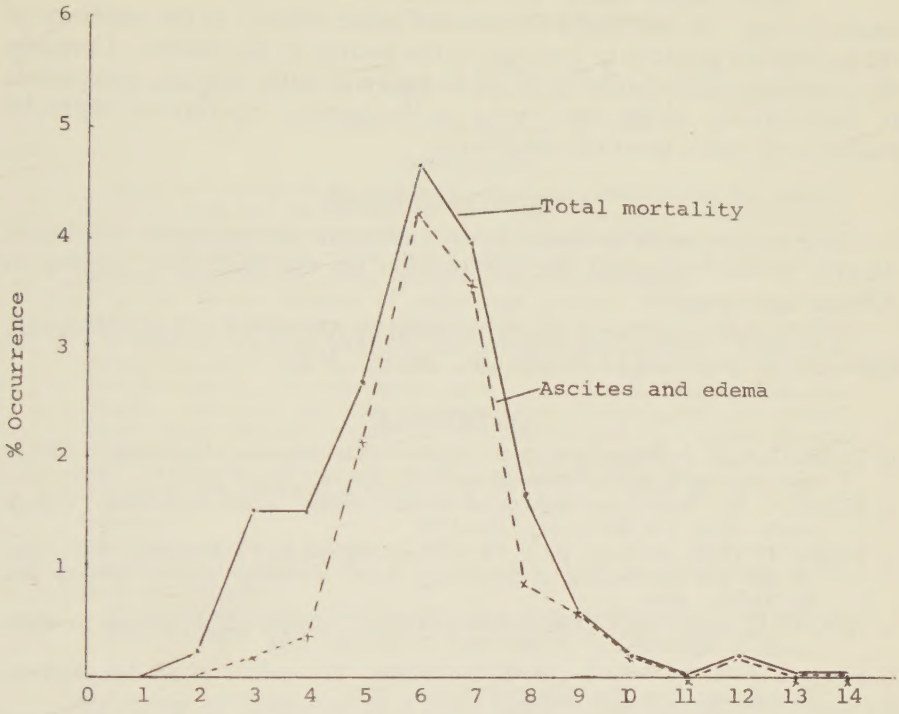

FIGURE 1. Pattern of mortality and occurrence of ascites and edema in relation to days on test.

lower in the groups receiving 4500 and 6000 p.p.m. of salts in the water supply (Lots 23 and 24). No explanation for this effect can be offered.

It is interesting to note that 50 per cent of the poults survived even on the highest levels of salts in the feed and drinking water. It would, therefore, appear that individual poults are able to adjust their body mechanisms in such a way as to tolerate fairly high levels of salt in the feed and water supply.

The pattern of mortality and the occurrence of ascites and edema in relation to days on test is shown in Figure 1. It can be seen that level of mortality and incidence of ascites and edema increased sharply after 3 days of treatment, reached a peak during the sixth and seventh days and declined sharply and stopped after 12 days.

The results obtained lend some support to the hypothesis that ascites and edema, under field conditions, may be caused by the levels of salts present in the water and/or feed supplies. Some farm water supplies contain fairly high concentrations of sodium salts which, if combined with high levels of salt in the feed, might lead to the appearance of the disorder. Levels of salt comparable to those used in the experimental rations might be encountered in the field if separation of salt occurred in the feed during mixing or feeding. Bigland (2) observed that the salt content of feed taken from feeders was often considerably higher than that of the same feed taken

from the bag. He attributed the increase in salt content to the tendency of the heavier salt particles to gravitate to the bottom of the feeder. Certainly the possibility exists that levels of salt in feed and water supplies, comparable to those causing ascites and edema in the present experiment, might be encountered under practical conditions.

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THE DETERMINATION OF CHROMIC OXIDE IN SAMPLES OF FEED AND EXCRETA BY ACID DIGESTION AND SPECTROPHOTOMETRY

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ABSTRACT

Numerous aspects of the wet acidic digestion procedure for the assay of Cr_2O_3 in feed and excreta samples have been investigated; these included time and temperature of heating, the effects of sulphuric acid concentration, and the wavelengths at which optical densities are read. On the basis of the findings, modifications of the procedure have been adopted.

As the optical densities of dichromate solutions, which are the end products of the digestion, are influenced by their sulphuric acid molarities, it is essential that such molarities be kept relatively constant. Reading of optical densities at 350 mμ permits a much greater sensitivity in the assay than does the use of a wavelength of 444 mμ. Standard curves with correlation coefficients of 1.000 between Cr_2O_3 concentration and optical density were obtained consistently, demonstrating a high degree of precision. When a 3-gram sample of feed and a 1-gram sample of excreta are employed, the observed ratio $\frac{Cr_2O_3 \text{ per gm. feed}}{Cr_2O_3 \text{ per gm. excreta}}$ must be corrected as follows:

Corrected ratio = (observed ratio $\times$ 1.110) $-$ 0.045. This correction accounts for the background light absorption at 350 mμ attributable to the feed and excreta.

The modified method was found to give comparable results to the sodium fusion technique with evidence of slight advantages over the latter.

INTRODUCTION

A number of indicators have been employed in digestibility studies to avoid the necessity for the total collection of faecal material. The most widely used of such indicators, especially in studies with monogastric animals, has been chromium sesquioxide (Cr_2O_3). The use of this indicator has been reported to allow the derivation of more precise digestibility data than was possible when total collection was employed (6, 14). Moore (12) has discussed the advantages and disadvantages of the use of Cr_2O_3 as an indicator of digestibility.

There are many methods available for determining the Cr_2O_3 content of feed and excreta samples. Preparation of solutions of the Cr_2O_3 contained in such samples has been achieved by various procedures of which wet digestion with acid[1] (2, 9, 11), ashing followed by alkali fusion[2] (1, 3, 6, 10, 13), and ashing followed by acid digestion (5) are perhaps the most widely accepted. Once in solution the Cr_2O_3 is usually estimated photometrically although other procedures have been employed.

While determining the Cr_2O_3 content of feed and excreta samples by a wet digestion procedure (9) evidence was obtained to suggest that the accuracy of the method might be improved by certain modifications. Day (7), who employed a digestion mixture of sulphuric acid, perchloric acid and sodium molybdate, reported that time, temperature and acidity of digestion are critical factors in the Cr_2O_3 assay. Kimura and Miller (11) varied the time of digestion, the amount of perchloric acid and the method of cooling the digests and found that none of these factors influenced the re-

[1]Renner, R. *Private communication.* 1959.
[2]Titus, H. W. *Private communication.* 1959.

covery of chromic oxide; however, these latter workers did not employ sulphuric acid in the digestion mixture which may explain the apparent contradiction between their findings and those of Day (7).

The present report concerns a number of experiments designed to study various phases of the acid digestion procedure for the assay of Cr_2O_3 in feed and excreta samples. A comparison between the acidic digestion and alkali fusion technique was also made. Some of the experimental findings confirm previously demonstrated properties for chromium compounds and ions but are included herein because much of this information is not readily available to many nutritionists. Further, as these findings were the basis for the adoption of a number of modifications to the assay procedure used by Hill and Anderson (9) it seemed necessary to include them.

MATERIALS AND METHODS

In order to avoid the necessity for frequent reference to the original report a stepwise summary of the method used by Hill and Anderson (9) is presented. This method was basic to most of the experiments reported herein.

Reagents

Concentrated nitric acid

Digestion mixture: 10 grams sodium molybdate are dissolved in 150 millilitres distilled water; 150 millilitres concentrated sulphuric acid are added slowly and the solution is cooled in an ice bath; 200 millilitres 70 per cent perchloric acid are added slowly with frequent stirring.

Equipment

100-millilitre Kjeldahl flasks calibrated to 110 millilitres.

Beckman Model D.U. spectrophotometer.

Procedure

1) Weigh sample, containing approximately 10 milligrams Cr_2O_3, wrap in filter paper and transfer to 100-millilitre Kjeldahl flask.
2) Add 10-millilitres concentrated nitric acid and allow to stand overnight.
3) Heat to dryness over an open flame using a microburner.
4) Add 15 millilitres digestion mixture and digest over an open flame until green colour changes to yellow, orange or red.
5) Chill flask, add 90 millilitres distilled water, cool, bring flask contents to volume (110 millilitres) with distilled water.
6) Allow to stand overnight to precipitate inorganic material.
7) Read optical density at 430 mμ.

When the above method was applied in this laboratory it was found that chilling the flasks the moment the digests changed in colour from green to yellow was not satisfactory because a reversal in colour change frequently resulted. It was, therefore, decided to extend the digestion period for 15 minutes following the colour change; in addition, the chilling of the flask following digestion was omitted. This modification was employed in all studies reported herein unless stated otherwise.

EXPERIMENTAL

Spectrophotometric Properties and Stability of Cr_2O_3 Digests

It has been suggested* that the above method may be improved by replacing 20 millilitres of the water, added during step 5 with concentrated sulphuric acid and by reading the optical density of the final solution at 444 mμ.

*Renner, R. *Private communication.* 1959.

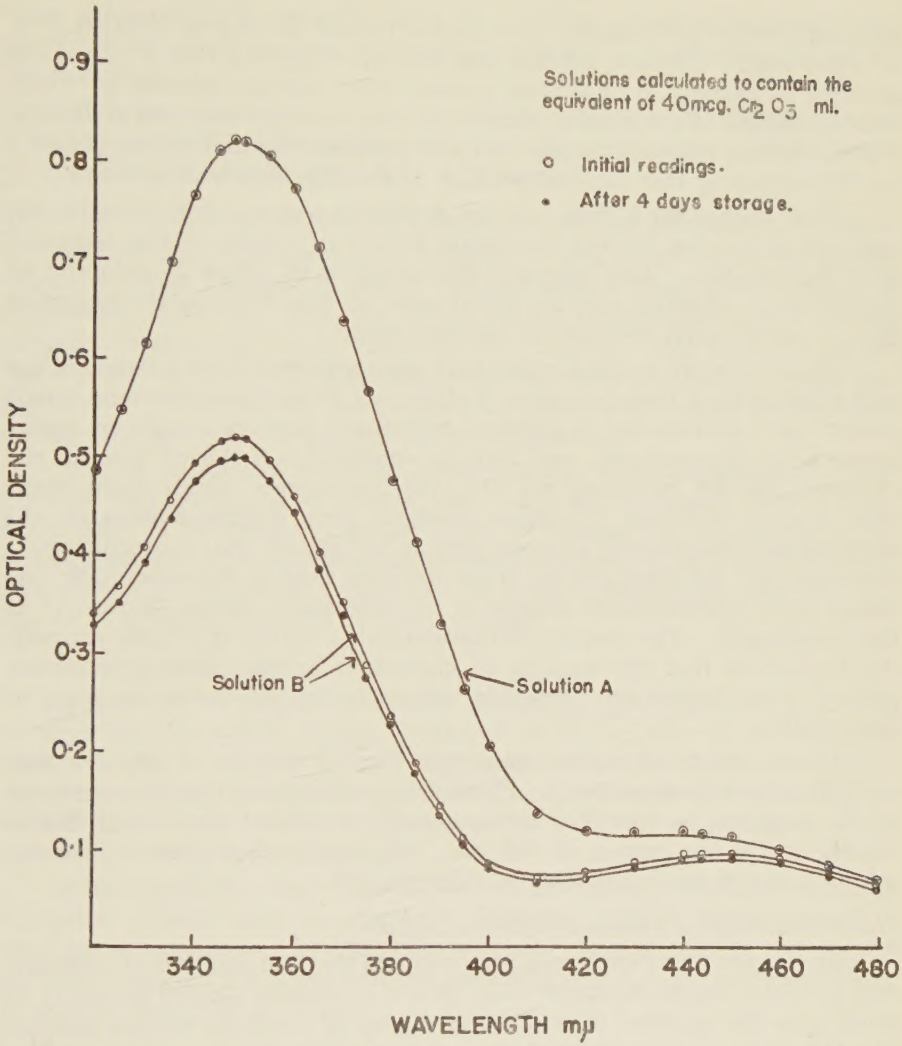

FIGURE 1. Absorption spectra of solutions prepared by the wet acid digestion of Cr_2O_3.

The spectral absorption curves of solutions obtained by oxidizing Cr_2O_3 by both the original procedure (A) and the same procedure modified by the addition of sulphuric acid (B) were determined on the freshly-prepared solutions and on the same solutions after storage for 4 days. As 20 per cent sulphuric acid was found not to absorb light over the wavelength range studied, distilled water was employed as a blank.

Results and Discussion

The absorption spectra obtained are presented in Figure 1. It will be seen that both solutions gave a pronounced peak at 348 mμ and a much less pronounced peak at about 444 mμ. There was no peak at 430 mμ; consequently, it was deemed unnecessary to investigate the optical densities of

solutions at this wavelength. The higher optical density of solution A at all wavelengths between 325 mμ and 460 mμ suggested that it was more sensitive to changes in chromium content than was the solution to which acid was added (B) when the criterion of measurement was optical density. The absorption curves are similar to those published for dichromate ($Cr_2O_7^=$) (4, 8), suggesting that the end product of the digestion is dichromate.

After storage for 4 days, solution A yielded a curve identical with that obtained on the first day but the optical density of solution B was decreased at all wavelengths. This suggested that solutions to which 20 millilitres of concentrated sulphuric acid are added prior to final dilution are less stable than those to which no addition has been made.

This is contrary to what might have been expected if the purpose of the acid addition were the prevention of shifts in a dichromate-chromate equilibrium. It is known that in solution dichromate ions are usually in equilibrium with chromate ions, and that the equilibrium is shifted towards the chromate side by increasing the pH (16), an increase which might result from aqueous dilution. Chromate solutions have spectrophotometric absorption curves exhibiting a strong peak at 375 mμ (8, 16); consequently, if chromate ions had been present in the solutions used in this experiment one would have expected some change in the absorption curves (Figure 1) at this wavelength. The absence of changes in the curves at 375 mμ supports the hypothesis that the amount of chromium in the form of chromate present in the digests was negligible relative to the amount in the form of dichromate.

The results of this experiment suggest that the addition of sulphuric acid to Cr_2O_3 digests is unnecessary. Further it would appear that the sensitivity of the assay can be improved by measuring the optical densities of diluted digests at 350 mμ instead of 444 mμ. No support was obtained for the selection of 430 mμ as the assay wavelength (9).

H_2SO_4 Molarities of Feed and Excreta Digests

As the previous experiment demonstrated that the addition of sulphuric acid to Cr_2O_3 digests depressed their optical densities it seemed necessary to investigate the variation in acidities of digests of feed and excreta samples.

When determining Cr_2O_3 in digestibility studies 3-gram samples of feed and 1-gram samples of excreta are generally used. Six samples of chicken feed, six samples of chicken excreta and eight samples of chromium oxide were digested and the resulting solutions were diluted to 500 millilitres with water. Aliquots of the diluted solutions were titrated with 0.1142 N sodium hydroxide. The resulting data were expressed in terms of sulphuric acid molarity. Dilution of the digests to 500 millilitres instead of 110 millilitres was necessary because the optical densities of the more concentrated digests were too great to read at 350 mμ.

Results and Discussion

Six feed samples when digested and made up to 500 millilitres were found to have a mean molarity with respect to sulphuric acid of 0.180 (range 0.172-0.190) whereas an equal number of samples of chicken excreta gave a

mean value of 0.189 (range 0.183-0.194). Eight solutions resulting from the oxidation of Cr_2O_3 alone had a mean molarity of 0.189 when diluted to 500 millilitres.

Since the completion of this investigation many other diluted digests have been titrated. The molarities of such digests in terms of H_2SO_4 have always fallen within the range 0.16 to 0.20 molar.

Effect of H_2SO_4 Concentration on Optical Densities of Dichromate Solutions

The present experiment was designed to study the magnitude of the effects of small changes in acidity on the optical densities of potassium dichromate solutions. The use of potassium dichromate instead of the oxidation product of Cr_2O_3 was dictated by the fact that solutions of the latter always contain some sulphuric acid. Cardone and Compton (4) and Englis and Wallerman (8) have reported a decrease in optical density associated with increasing sulphuric acid concentration.

A stock solution of potassium dichromate in water was prepared. Aliquots of this solution were diluted with water and sulphuric acid to form eight pairs of solutions, each calculated to contain the equivalent of 20 micrograms Cr_2O_3 per millilitre but differing in sulphuric acid molarity from zero to one. The optical densities of the solutions were determined on the day of preparation and after 2 days' storage.

Results and Discussion

A summary of the optical density data is presented in Table 1. It will be seen that the optical density decreased as the molarity of the solutions, with respect to sulphuric acid, increased. The differences in optical densities resulting from the range of acid molarities encountered in digests of feed and excreta (*see page 170*) are not of sufficient magnitude to introduce major errors in the Cr_2O_3 assay.

It was noted that some of the solutions showed an appreciable decrease in optical density after storage for 2 days, the decrease being greatest at the highest molarities. At a molarity of 0.20, which is close to the average molarity observed for samples in the preceding study, there was no decrease in optical density after storage.

TABLE 1. — THE INFLUENCE OF SULPHURIC ACID ON THE OPTICAL DENSITIES OF POTASSIUM DICHROMATE SOLUTIONS[1]

Molarity[2]	O.D. day of preparation	O.D. 2 days later
0.00	0.422	0.422
0.05	0.403	0.398
0.10	0.388	0.384
0.15	0.378	0.373
0.20	0.368	0.368
0.25	0.360	0.358
0.30	0.358	0.347
1.00	0.299	0.290

[1]Wavelength 350 mμ

[2]Expressed in terms of sulphuric acid

The Time of Digestion

Hill and Anderson (9) advocated that the digestion of samples be terminated when they change in colour from green to yellow, orange or red. In this laboratory digestion has been continued for 15 minutes following the colour change to ensure complete oxidation. Day (7) found that time of digestion was a critical factor when sulphuric : perchloric acid mixtures were employed, whereas Kimura and Miller (11) reported that time of digestion did not affect Cr_2O_3 recoveries when perchloric acid was the only acid used.

Three feeds and two samples of excreta were digested for varying periods of time following the change in colour from green to orange. The digests were then diluted to 500 millilitres with distilled water and their optical densities measured at 350 mμ.

Results and Discussion

The mean results of this experiment are presented in Table 2. It is apparent that the optical densities of the solutions removed immediately following the colour change were lower than those of the solutions allowed to digest for a longer period. Solutions digested for 5 to 30 minutes following the colour change had comparable optical densities. Analysis of variance confirmed this interpretation. In view of these findings, digestion for 15 minutes following the change in colour was retained.

Temperature of Digestion

Day (7) stressed that the temperature of digestion can influence the optical densities of Cr_2O_3 digests. Hill and Anderson (9) employed microburners to heat their digestion flasks and made no comment concerning temperatures. In this laboratory a micro-Kjeldahl digestion unit* with electrically-heated hot plates (150 watts each) controlled by 11-position rheostats has been employed. Prior to this study rheostat settings of 0 (lowest heat) and 3 were used for the oxidation of 3-gram samples of feeds and

*Model 3802 Cole-Parmer Instrument and Equipment Co., Chicago, Ill.

TABLE 2. — THE EFFECT OF LENGTH OF TIME OF DIGESTION ON THE CONCENTRATION OF CHROMIUM IN THE DILUTED DIGEST AS MEASURED BY OPTICAL DENSITIES AT 350 mμ[1]

	Time in minutes[2]						
	0	5	10	15	20	25	30
Feed	0.313	0.425	0.440	0.434	0.427	0.418	0.419
Feed T-3-60	0.331	0.411	0.418	0.414	0.407	0.414	0.398
Feed C-2-60	0.342	0.390	0.402	0.392	0.392	0.395	0.389
Excreta T-3-60	0.319	0.337	0.338	0.331	0.335	0.341	0.340
Excreta C-2-60	0.452	0.477	0.479	0.477	0.471	0.472	0.475
Mean	0.351	0.408	0.415	0.410	0.406	0.408	0.404

[1]Each value represents the mean of three determinations.
[2]Time of heating after colour change from green to orange

1-gram samples of excreta, respectively. To test the influence of temperature on the optical density reading of digests a number of samples of a feed were digested at rheostat settings of 0, 3, 5 and 8, and the digests were diluted to 500 millilitres with water.

Results and Discussion

The mean optical densities, measured at 350 mμ, for the diluted digests were 0.440, 0.433, 0.422 and 0.434 for the rheostat settings of 0, 3, 5 and 8, respectively. Analysis of variance failed to reveal a significant difference in optical densities associated with rheostat settings. It would, therefore, appear that, although temperature variations may influence the Cr_2O_3 assay, such variations would have to be very large to introduce significant error into the results obtained by the digestion procedure described here. The rheostat settings employed previously in this laboratory were considered to be satisfactory and their use was retained.

The Use of Sulphuric Acid in the Digestion Mixture

Day (7) has criticized the use of mixtures of perchloric acid and sulphuric acid because of her observations that time of heating with this mixture must be carefully controlled in order to avoid errors due to overheating. Smith (15) has stated that mixtures of the two acids will yield traces of hydrogen peroxide at temperatures of 180 to 185°C. and, consequently, may have reducing as well as oxidizing properties.

An attempt was made to compare perchloric acid alone with the perchloric acid : sulphuric acid mixture in the digestion of a number of samples of feed and excreta. The amount of catalyst, sodium molybdate, used was the same in all cases.

Results and Discussion

The digestion reaction was quite violent when perchloric acid alone was used, so much so in the case of feed samples that all of these were lost. Although the digestion of most of the samples of excreta with perchloric acid was completed successfully yielding extracts with an average optical density appreciably higher than that of the extracts from the perchloric acid: sulphuric acid mixture (0.494 compared to 0.449), the variability of the optical densities obtained with the single acid was considerably greater.

It is recognized that the presence of sulphuric acid slows down and moderates the digestion reaction and that the omission of this acid may necessitate smaller amounts of catalyst, as used by Kimura and Miller (11), for successful results. In view of the satisfactory reproducibility and stability obtained with the acid mixture containing sulphuric acid in this laboratory, however, it was decided to continue the use of this type of digestion mixture.

Standard Curves at Two Wavelengths

Standard curves for Cr_2O_3 were prepared at both 350 mμ and 444 mμ. A weighed amount of Cr_2O_3 was wrapped in a filter paper, digested and diluted to 500 millilitres with distilled water. Aliquots of this solution were

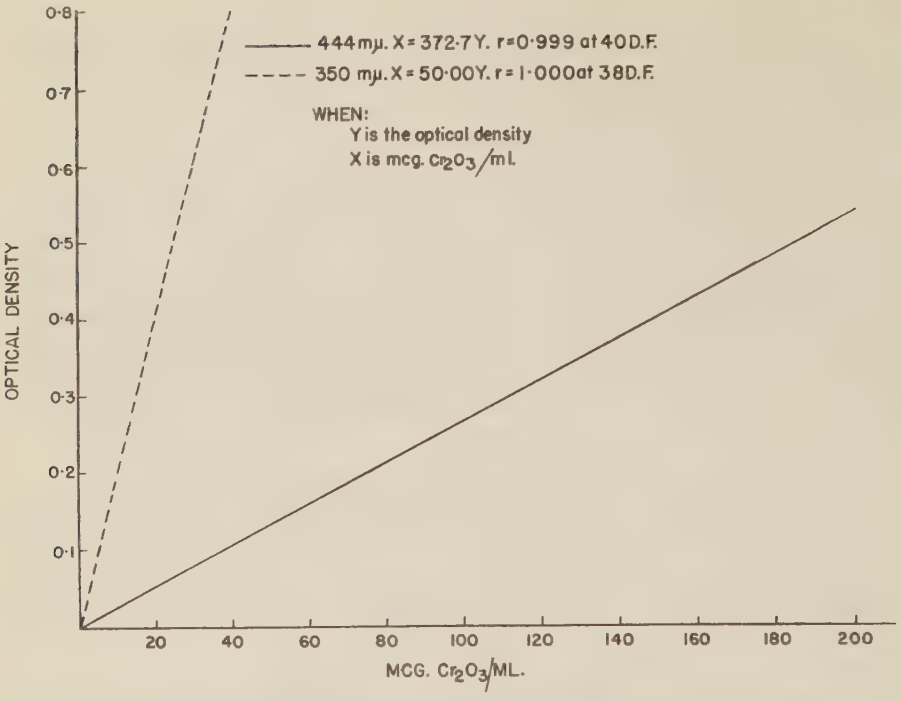

FIGURE 2. Standard curves for Cr_2O_3 demonstrating the improved sensitivity obtained when a wavelength of 350 mμ is used in preference to 444 mμ.

diluted with 0.18 molar sulphuric acid to provide ten solutions of different chromium content. The greater sensitivity of the method employing a wavelength of 350 mμ relative to that at 444 mμ made it desirable to prepare different primary solutions for each of the standard curves.

Further standard curves were obtained at 350 mμ using five more preparations of the digestion mixture in order to determine the precision with which the results could be repeated.

Results and Discussion

Two representative standard curves are presented in Figure 2. Each curve is based on the readings of four solutions of each concentration prepared from four separate Cr_2O_3 digests. It is apparent that a small change in the chromium content of the solution is more likely to be detected at 350 mμ than at 444 mμ. It is usual to digest a 3-gram sample of feed containing 0.3 per cent Cr_2O_3; such a quantity of Cr_2O_3 necessitates dilution of the digest of 500 millilitres if the optical density readings are to fall within a satisfactory range.

It was found that for seven standard curves the correlation coefficients, relating the optical densities to the chromium concentrations, were all equal to 1.000 and the slopes of the lines were identical. These findings reveal the

high reproducibility of results and add further support for the use of sulphuric acid. As the standard curves determined at 350 mμ were linear it would appear that, within the range of concentrations studied (0-40 μg. Cr_2O_3 per ml.), solutions of a digest of Cr_2O_3 obey Beer's Law. It should be emphasized that it is necessary to prepare and dispense the digestion mixture with an accuracy sufficient to ensure approximate uniformity of acid concentration in the final solutions of the digests.

Correction for Background Light Absorption

It had been observed that digests, prepared in the usual way from feed and excreta samples containing no Cr_2O_3 and diluted to the customary 500-millilitre volume, exhibited light absorption at 350 mμ. It was apparent that this background absorption would introduce errors into determined Cr_2O_3 values and into the values for the ratio $\frac{Cr_2O_3 \text{ per gm. feed}}{Cr_2O_3 \text{ per gm. excreta}}$ which is the desired result of digestibility studies using the Cr_2O_3 indicator method. It became necessary, therefore, to attempt to derive a correction for such errors.

Feeds with added Cr_2O_3 and samples of the corresponding faeces were subjected to the analytical procedure for Cr_2O_3 as were samples of the same feeds without Cr_2O_3 and of the corresponding faeces. Cr_2O_3 ratios for the former were calculated using Cr_2O_3 values obtained by means of the regression equation derived in the preceding study, and using the same values corrected for the background absorption observed for the corresponding Cr_2O_3-free feed or faeces. Five species (chicken, guinea pig, rat, rabbit and mink) were used in this study and a total of 38 Cr_2O_3 ratios were obtained by each of the above procedures. The data were subjected to regression analysis.

Results and Discussion

The results of this experiment are expressed graphically in Figure 3. The correlation between the corrected and observed ratios ($r = 0.997$ at 36 degrees of freedom) suggests that the derived regression equation, $Y = 1.110X - 0.045$, may be used to correct the Cr_2O_3 ratios (Y is the corrected and X is the observed ratio).

A Comparison of Chromium Oxide Ratios Derived by the Acid Digestion and Sodium Fusion Procedures

The acid digestion method of Hill and Anderson (9), modified in the light of the foregoing findings, was compared with the sodium fusion method of Brisson (3) as modified by L. L. Nesbitt in the laboratory of Titus*. The latter modification involves the measurement of optical densities at 375 mμ, the peak of the spectral absorption curve for chromate ions, instead of at 400 mμ.

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*Titus, H. W. *Private communication.* 1959.

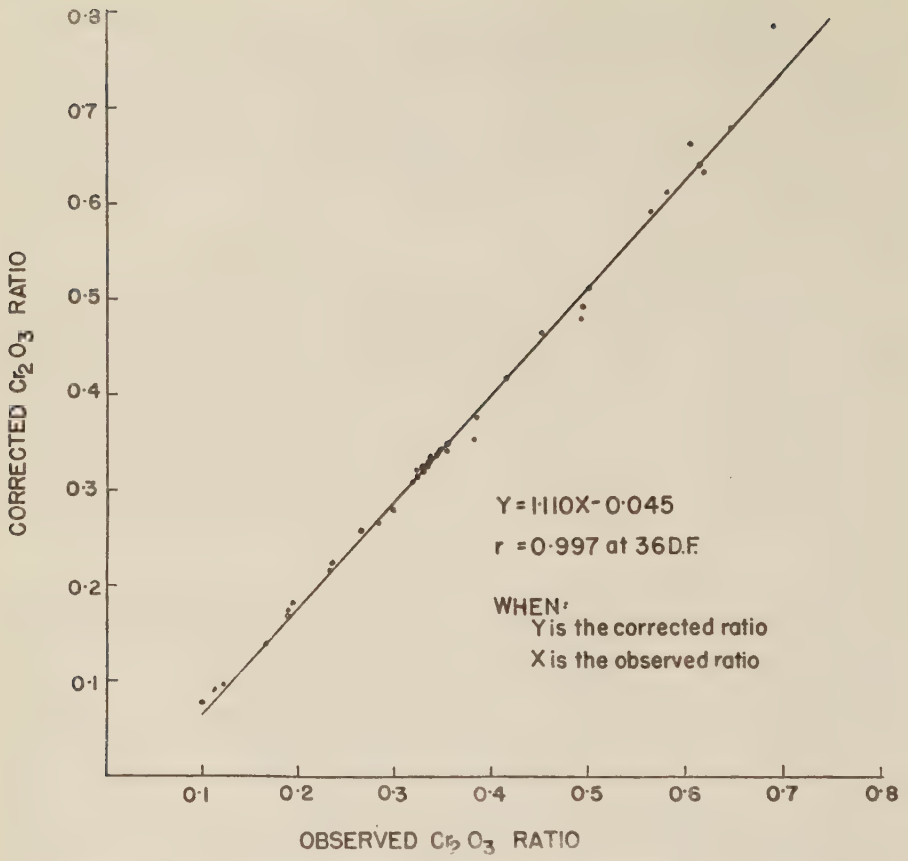

FIGURE 3. The relationship between observed and corrected Cr_2O_3 ratios.

A standard curve at 375 mμ was established using 10 levels in triplicate of Cr_2O_3 treated by the fusion method; water was used as a blank for the spectrophotometric readings. Five pairs of samples of poultry feed and related excreta were assayed by both the acid digestion and sodium fusion methods and chromium oxide ratios were calculated.

Results and Discussion

The standard curve obtained by the sodium fusion technique had the form:

$$X = 15.427Y + 0.033$$

where X was the Cr_2O_3 content of the sample (mg.), and Y was the optical density at 375 mμ. Brisson (3) derived a standard curve at 375 mμ which had the form $X = 15.97Y$.

The mean Cr_2O_3 ratios were: 0.419 (0.420), 0.270 (0.286), 0.384 (0.371), 0.272 (0.275) and 0.245 (0.252), where the figures in parentheses are the means of values derived by the fusion procedure. Analysis of variance failed to reveal any significant difference between the ratios derived by the two procedures.

TABLE 3. — THE RECOVERY OF Cr_2O_3 FROM FEED

Cr_2O_3 added to feed	Recoveries					
	Acid digestion		Sodium fusion uncorrected		Sodium fusion corrected[1]	
mg./gm.	mg./gm.	%	mg./gm.	%	mg./gm.	%
0.33	0.34	103	0.52	158	0.32	97
1.00	0.96	96	1.26	126	1.05	105
1.67	1.62	97	1.86	111	1.66	99
2.00	1.98	99	2.10	105	1.89	94
2.33	2.34	100	2.56	110	2.35	101
2.67	2.66	100	2.83	106	2.62	98
3.00	3.01	100	3.25	108	3.04	101
3.33	3.29	99	3.42	103	3.21	96
4.00	4.03	101	4.14	104	3.94	98
Mean[2]		99.4		107.9		98.7

[1]Corrected for background light absorption

[2] $\left(\frac{\Sigma \text{ mg. } Cr_2O_3 \text{ recovered}}{\Sigma \text{ mg. } Cr_2O_3 \text{ added to feed}}\right) \times 100$

The Recovery of Cr_2O_3 by Both the Acid Digestion and Sodium Fusion Techniques

It was of interest to compare the recovery by the two assay methods (acid digestion and sodium fusion) of known amounts of Cr_2O_3 added to feed. A sample of poultry feed was divided into ten portions to which Cr_2O_3 was added at levels of from 0 to 4 milligrams per gram as shown in Table 3. The Cr_2O_3 content of each of the resulting feeds was determined in duplicate by both the acid digestion technique as modified in the preceding studies and by the sodium fusion technique*. Because of the background light absorption at 350 mμ of acid digests of feed, the diluted digest of the chromium-free feed was used as a spectrophotometric blank in the acid digestion assay.

Results and Discussion

It was found that the chromium-free feed, subjected to sodium fusion, yielded a diluted digest which had an optical density of 0.038 at 375 mμ (average of five determinations, range 0.025 to 0.054); consequently, the chromium contents of the test feeds determined by the fusion technique were calculated using both water and chromium-free digest as blanks. The results of the assays are presented in Table 3.

It appears that the modified acid digestion procedure is somewhat more accurate than the sodium fusion technique when the criterion of evaluation is Cr_2O_3 recovery. The corrected sodium fusion method (i.e., using a chromium-free digest as blank) yielded results comparable to those obtained by acid digestion. In view of these findings, it would seem that the acid digestion procedure (as modified) is to be preferred for the determination of Cr_2O_3 ratios, at least until a correction equation, similar to that described (*see page 175*), is derived for the fusion technique.

*Titus, H. W. *Private communication.* 1959.

CONCLUSIONS

It is proposed that the Cr_2O_3 assay of Hill and Anderson (9) should be modified in the following respects:

1) Digestion should be continued for 15 minutes after the colour changes from green to orange to ensure constant acidity and maximum colour development.
2) Chilling of the flask on completion of digestion may be omitted.
3) Digests should be diluted to 500 millilitres with water before being allowed to stand overnight.
4) In the preparation of standard curves a weighed quantity of Cr_2O_3 should be digested, the digest diluted to 500 millilitres with water, and appropriate dilutions prepared therefrom with 0.18 molar sulphuric acid.
5) Optical densities should be read at 350 mμ.
6) Calculated chromic oxide ratios $\frac{(Cr_2O_3/\text{gm. feed})}{(Cr_2O_3/\text{gm. excreta})}$ should be corrected for background light absorption at 350 mμ by using the equation $Y = 1.110\,X - 0.045$ (Y is the corrected and X the observed ratio).

Contrary to some reports, the use of sulphuric acid along with perchloric acid in the digestion mixture was found to be highly satisfactory, as judged by high reproducibility of results and stability of the digests. A proposed addition of sulphuric acid following digestion was found to be unnecessary.

Although no significant difference was found between ratios determined on the same feed and excreta samples by the acid digestion and the sodium fusion techniques, recovery of added Cr_2O_3 was better with the acid digestion procedure. It appears, furthermore, that a correction for background light absorption at 375 mμ is needed for the sodium fusion method.

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EFFECT OF AGE AT WEANING OF SHROPSHIRE LAMBS ON WEIGHT GAINS AND CARCASS SCORE

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ABSTRACT

Differences in the rate of gain from birth to 140 and 170 days of age for lambs weaned on grass-white clover pasture, at average ages of 10, 15 and 20 weeks, were not significant ($P < 0.05$) as measured by the linear and quadratic regressions. The average weights at weaning were 39, 54 and 66 pounds respectively. The rates of gain of the lambs were affected by type of birth, sex and year of rearing.

The age at weaning, type of birth and year of rearing had insignificant effects on the commercial grades of the carcasses from wether lambs.

None of the interaction effects for any of the response criteria studied reached statistical significance.

The results indicate that certain advantages from early weaning of lambs on pasture with ample immature forage were realized without adversely affecting the performance of carcass grades.

INTRODUCTION

Clarke (4) estimated that the efficiency of converting grass to lamb via ewe's milk was approximately 9 per cent (for lambs from 3 to 4 months of age) whereas the direct conversion of grass to lamb was about 30 per cent. Other possible advantages for early weaning of lambs have been reported (1, 3, 4, 13). Recent research (13) has shown that, at about 8 weeks of age, grazing lambs can digest forage with the efficiency of an adult. Baird *et al.* (1) found that most of the milk production of ewes had ceased by 15 weeks after lambing and Clark[3] found that for ewes nursing twins the milk production of the first week of the lactation was approximately double the production of the eighth week. Dickson (6) has suggested that after 2 months of age grass becomes the dominant factor in the lamb's diet. The performance of lambs weaned at 6 weeks of age in drylot compared favourably with those weaned at 9 weeks in experiments reported by Hinds *et al.* (8). Conversely, Baird *et al.* (1) found that early-weaned lambs made slower gains than unweaned controls. Stage of maturity of the pasture growth is an important factor in the performance of early-weaned lambs (13).

The present paper records the rate of gain from birth to 140 and 170 days of age and the scores and commercial grades for the carcasses of purebred Shropshire lambs weaned on grass–white clover pasture at 10, 15 and 20 weeks of age.

MATERIALS AND METHODS

Animals

All the lambs born from 1956 to 1958 inclusive from approximately 60 mixed-age purebred Shropshire ewes each mated to open-faced British-type purebred Shropshire rams were used in this study. The flock was wintered indoors and fed good quality grass-legume hay and silage. Approximately 4 weeks prior to lambing one-half pound of a concentrate

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TABLE 1. — DESIGN OF THE EXPERIMENT

Year	Sex	Age at weaning (weeks)					
		10		15		20	
		Single	Twin	Single	Twin	Single	Twin
1956	M*						
	F						
1957	M						
	F						
1958	M						
	F						

*Males were castrated at 10-14 days old.

mixture (equal weights of whole oats and wheat bran) per ewe was added to the daily ration until the ewes and lambs were put out to pasture when the lambs were approximately 9 weeks old. The flock had ready access to mineral (equal weights of iodized-cobaltized salt and feeding bone meal) and water supplies. The ewes and lambs were run as a single flock until the lambs were weaned. All ewes were treated for internal parasites, using phenothiazine. At birth all lambs were weighed and identified, and allotted according to sex and type of birth in accordance with the experimental design shown in Table 1.

All lambs were docked and the male lambs were castrated when 10 to 14 days of age. The lambs were weighed and weaned on the Wednesday of the week nearest the age of weaning for each group and were weighed individually at 28-day intervals from weaning to the time of marketing.

All wether lambs were slaughtered when they reached a minimum live weight of 80 pounds and the degree of finish required for top carcass grade as estimated by handling of the animals. The carcasses were classified for conformation, finish and fleshing of the leg, loin, neck and shoulder, and for blockiness. Commercial grades were obtained for all carcasses. Numerical values from 1 to 3 were assigned for blockiness and commercial grade and 1 to 4 for all other carcass characteristics with 3 and 4 being the highest scores, respectively.

Pastures

The pastures consisted of a well-established sward of grasses and white clover. The weaned lambs rotationally grazed one 3-acre and one 1.5-acre adjacent fields. The ewes and unweaned lambs rotationally grazed two fields located in the same general area as those grazed by the weaned lambs. The larger field grazed by the weaned lambs received annual applications of 0-20-20 fertilizer and ammonium nitrate at the rate of 300 and 100 pounds per acre, respectively. All other fields received an annual application of 3-15-6 fertilizer at the rate of 300 pounds per acre. The excess forage was

TABLE 2. — MEAN BIRTH AND WEANING WEIGHTS AND WEIGHT GAINS, FROM BIRTH TO 140 AND 170 DAYS OF AGE, FOR SHROPSHIRE LAMBS WEANED AT 10, 15 AND 20 WEEKS OF AGE
(Based on the mean of 4 lambs per cell)

Comparison	No. of lambs	Mean birth weight	Mean weaning weight	Mean weight gain/day	
				From birth to 140 days of age	From birth to 170 days of age
		lb.	lb.	lb.	lb.
All lambs	144	7.9	53.0	0.39	0.38
Age at weaning					
10 weeks	48	8.1	38.8	0.37	0.37
15 weeks	48	7.9	54.2	0.39	0.38
20 weeks	48	7.8	66.2	0.41	0.39
Type of birth					
Singles	72	9.0*	57.0	0.41*	0.40*
Twins	72	6.9	49.1	0.37	0.37
Sex					
Wethers	72	8.3	55.9	0.41*	0.40*
Ewes	72	7.6	50.2	0.37	0.36
Year of rearing					
1956	48	8.1	53.4	0.36*	0.35*
1957	48	8.3	53.7	0.39	0.37
1958	48	7.4	52.0	0.42	0.42

*Significant ($P < 0.05$)

TABLE 3. — ANALYSIS OF VARIANCE PLANS AND MEAN SQUARES FOR AVERAGE DAILY GAINS, MARKET WEIGHT AND DRESSING PERCENTAGE FOR SHROPSHIRE LAMBS

Source of variance	Degrees of freedom	Mean square variance	
		Average daily gain	
		From birth to 140 days of age	From birth to 170 days of age
1. For lamb gains (4 lambs per cell)			
Total	35		
Years	2	0.0411*	0.0701*
Treatments	11	0.0135	0.0127
Weaning age—linear regression component	1	0.0173	0.0098
—quadratic regression component	1	0.0000	0.0006
Type of birth	1	0.0560*	0.0403*
Sex	1	0.0685*	0.0836*
Interactions	7	0.0068	0.0057
Remainder (years x treatments)	22	0.0037	0.0034
2. For carcass characteristics (4 wether lambs per cell)		*Market weight*	*Dressing percentage*
Total	17		
Years	2	138.01*	9.48
Treatments	5	38.79	23.11
Weaning age	2	4.50	25.93*
Type of birth	1	181.77*	58.32*
Interaction	2	0.80	2.69
Remainder (years x treatments)	10	18.78	4.17

*Significant ($P < 0.05$)

grazed by cattle when the growth of forage exceeded the needs of the ewes and/or lambs. When all lambs were weaned they received a concentrate mixture made up of 90 parts of oats and 10 parts of linseed oilmeal, by weight, at the rate of one-half pound per lamb daily. Water and iodized-cobaltized salt were supplied *ad libitum* on all fields.

Data Analysis

The data were subjected to analysis of variance according to the methods of Snedecor (12). The data presented are based on the mean of 4 lambs per cell. Tests of significance of the F values with 1 degree of freedom each for the data on birth weight and gains were made according to the sequential F test by Nair (10).

Carcass data of wethers only were used since the numbers of carcasses from ewes for each cell were so variable as to give different weighting effects to the means when included.

Statements with reference to statistical significance are made at the 5 per cent level of probability.

RESULTS AND DISCUSSION

Rate of Gain

The mean birth and weaning weights and main effects of age at weaning, type of birth (singles and twins), sex and year of rearing on the average daily gains of the lambs, from birth to 140 and 170 days of age, are presented in Table 2.

The analyses of variance data are shown in Table 3 with mean squares for gains, market weight and dressing percentage. No significant interactions were obtained for any of the response criteria studied.

There was no significant difference in weight gains, from birth to 140 and 170 days of age, between lambs weaned on grass–white clover pasture at average weights and ages of 39 pounds and 70 days, 54 pounds and 105 days and 66 pounds and 140 days, respectively, as measured by the linear and quadratic regression components (Table 3). These results are in agreement with the studies of Brothers and Whiteman (3) which indicated that lambs on wheat pasture and creep feed may be weaned when they reach both minimum weight and age of 50 pounds and 70 days, respectively, without adversely affecting their subsequent performance. Wardrop *et al.* (13) have also reported little difference in the performance of lambs weaned on immature pasture forage at 7, 10, 13 and 18 weeks of age.

There was no evidence of an adverse effect from early weaning of lambs during the immediate post-weaning period. The lambs weaned at 10 weeks of age made more rapid average gains in the first 2 weeks following weaning than during the remainder of the study, indicating that rumen function was developed sufficiently for the digestion of immature forage.

Age at weaning had no effect on death losses.

Single lambs made faster gains than twins, and wethers made more rapid gains than ewes. These results are in accord with numerous reports on the effects of type of birth and sex on the rate of lamb gains (2, 5, 7 and others).

TABLE 4. — MEAN MARKET WEIGHT, DRESSING PERCENTAGE AND SCORES FOR CARCASSES FROM SHROPSHIRE WETHER LAMBS, WEANED AT 10, 15 AND 20 WEEKS OF AGE
(Based on the mean of 4 lambs per cell)

Comparison	No. of lambs	Mean market wt.	Mean dressing percentag.	Mean score								
				Conformation	Blockiness	Finish	Fleshing	Leg	Loin	Neck	Shoulder	Commercial grade
		lb.										
All lambs	72	85.2	43.7	3.3	2.5	3.1	3.4	2.9	3.4	3.2	3.3	2.8
Age at weaning												
10 weeks	24	84.7	42.5*	3.2	2.3	3.0	3.1	2.7	3.2	2.9	3.0	2.7
15 weeks	24	85.4	44.3	3.3	2.6	3.2	3.3	3.1	3.4	3.3	3.5	2.8
20 weeks	24	85.4	44.3	3.5	2.7	3.2	3.6	2.9	3.5	3.4	3.6	2.9
Type of birth												
Singles	36	86.8*	42.8*	3.1	2.3	2.8*	3.1	2.7	3.1	3.1	3.1	2.7
Twins	36	83.6	44.6	3.5	2.7	3.4	3.6	3.1	3.6	3.4	3.6	2.9
Year of rearing												
1956	24	82.5*	43.5	3.1*	2.3	2.7*	3.1*	2.8*	3.1*	2.9	3.3*	2.8
1957	24	85.9	44.4	3.9	2.9	3.7	3.9	3.3	3.9	3.7	3.9	3.0
1958	24	87.1	43.1	3.0	2.3	3.0	3.0	2.7	3.1	3.2	2.8	2.6

*Significant ($P < 0.05$)

The age at weaning had little effect on the relative performance of single and twin lambs. Similar results for early-weaned single and twin lambs were obtained by Lewis *et al.* (9), however, Hinds *et al.** reported slower gains for twins than comparable single lambs weaned in drylot at 6 and 9 weeks of age.

The rate of gain was affected by the year in which the lambs were reared. This is in accordance with the work of Sidwell and Grandstaff (11), Blackwell and Henderson (2) and others, who have reported that the year of rearing lambs is an important source of variation in their body weight.

Carcass Characteristics

A summary of the effects of age at weaning, type of birth and year of rearing on the market weight, dressing percentage and carcass scores for wether lambs is given in Table 4.

Lambs weaned at 10 weeks had a lower dressing percentage than those weaned at 15 and 20 weeks of age. However, age at weaning had no significant effect on carcass scores. These findings are in general agreement with those reported by Baird *et al.* (1), Lewis *et al.* (9) and Wardrop *et al.* (13). However, Wardrop *et al.* (13) also reported that carcasses from early-weaned lambs were markedly inferior to those from late-weaned lambs when grazed on mature pasture forage.

Single lambs were heavier at market and had a lower dressing percentage than twins. The lower dressing percentage for single lambs was associated with a younger average age at slaughter and a significantly lower carcass score for finish, and lower average scores for all other carcass characteristics than those for twin lambs.

Year of rearing affected most of the carcass scores, however, these differences had no statistically significant effect on the scores for commercial carcass grade.

The results from the present experiment indicate that certain advantages from early weaning of lambs on pasture with ample immature forage were realized without adversely affecting the performance or carcass grades.

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RUMEN GAS ANALYSIS BY GAS-SOLID CHROMATOGRAPHY[1]

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ABSTRACT

Methods are described for sampling and analysing rumen gases. The analysis requires less than 15 minutes for the determination of hydrogen, oxygen, nitrogen, methane, carbon monoxide, carbon dioxide, and hydrogen sulphide, i.e., for all gases occurring in the rumen. The method is sensitive and requires only a small quantity of sample, and the sample volume need not be known. The presence of water or other vapours in the sample does not influence the results. Relative thermal detector responses have been determined for gases which occur in the rumen. These eliminate the necessity for the calibration of gas chromatographs using thermal detection. The first complete analysis of rumen gas is presented.

INTRODUCTION

Rumen gas is a product of rumen fermentation. Accurate and complete analyses are needed because the composition may be responsible for death in acute bloat (3, 4). The approximate composition is: carbon dioxide 67, methane 26, nitrogen 7, oxygen less than 1, hydrogen 0.2, and hydrogen sulphide 0.1 per cent by volume (2). In addition to these, carbon monoxide has been reported in amounts up to 0.17 per cent (4). As far as is known to the authors, there has not been an analytical method described which is capable of determining all components in a sample of rumen gas. The major components have been determined (9, 12) but by methods unsuitable for determination of minor components. Carbon monoxide (3, 4) and hydrogen sulphide (4, 9) have been determined, usually in large samples, but the other gases were not.

A review of the methods for gas analysis indicated that there was little hope of determining all the component gases by chemical absorption methods. However, gas-solid chromatography appeared to offer the possibility of determining all component gases relatively quickly, and in addition only small quantities of gas would be required. Therefore, it was decided to examine this method for analysis of rumen gas.

METHODS

Technique for Sampling Rumen Gas

The method of collecting rumen gas samples was a modification of that used by Washburn and Brody (12). The sample was collected in a 250-millilitre gas-collecting tube with a two-way stopcock at one end and a three-way at the other. A 3.5-inch, 13-gauge, B-D Yale hypodermic needle was connected to the two-way stopcock with a short length of rubber tubing and a 32-ounce polyethylene aspirator bottle was connected to the three-way stopcock with a 6-foot length of rubber tubing. Before taking a sample the apparatus was filled with water. To obtain a rumen gas sample, the needle was inserted into the upper rumen through the left abdominal wall, the aspirator bottle placed on the floor and the stopcocks opened to

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allow the water to drain into the aspirator bottle. When the gas-collecting tube was full of gas, the stopcocks were closed and the rubber tubing removed. With bloated cows it was not necessary to fill the gas-collecting tube with water because the rumen pressure could be utilized to flush and fill the tube with rumen gas. The composition of samples taken in this way did not differ in composition from duplicate samples taken by the water displacement method, which indicated that the water had no effect upon composition. When taking rumen samples, it was found most convenient for two operators to work together; one inserted the hypodermic needle and kept it in place, the other manipulated the aspirator bottle and stopcocks. In this way two operators sampled ten cows in 20 minutes.

Analyses of Rumen Gases

Instrumentation

The gas chromatograph used was a Beckman Model GC-2. The detector response was recorded with a Brown Electronik Potentiometer Recorder equipped with a Disc Chart Integrator Model K1-S. The chromatograph was fitted with 1.5- and 6-foot stainless steel columns, ¼-inch diameter, connected in parallel for alternate use (8, p. 36, fig. 32). The shorter column was packed with 28-200 mesh silica gel[1] and the 6-foot column with 5A molecular sieve[2].

The operating conditions for both analyses and the determination of the relative response factors reported below were as follows: helium carrier gas pressure 30 p.s.i.g.; filament current 350 ma.; column temperature 70°C.; and chart speed 0.5 inch per minute. Attenuations for gas analysis were: 1x for hydrogen, oxygen, carbon monoxide, and hydrogen sulphide; 5x for nitrogen; 10x for methane; and 50x for carbon dioxide.

Introduction of Gas Samples into the Chromatograph

Introduction of gas samples under water pressure into the gas chromatograph from the gas-collecting tube require approximately 100 millilitres of sample to flush the gas sampling valve. Furthermore, the gas remaining in the collecting tube differed in composition from the original, probably due to differences in solubilities of the component gases. Thus it appeared that the composition of gas in contact with water was not affected under partial vacuum but was affected under pressure. The modified gas chromatograph, described below, required only 6 millilitres of a gaseous rumen sample for each analysis. As many as ten aliquots have been drawn from the same tube with no discernible difference noted between analyses.

In order to reduce the amount of sample required, the gas chromatograph was modified as follows: The exhaust line of the gas sample valve was disconnected and the valve exhaust outlet capped so that the gas sample loop could be evacuated through the valve inlet. A small glass T was connected to the valve inlet. One arm of the T containing a stopcock was connected to a vacuum pump. The third arm was connected to the gas-collecting tube containing the sample. The sample was introduced by

[1]Cat. No. S-157, Fisher Scientific Co., Toronto, Ont.
[2]Cat. No. 9281, Podbielniak Inc., Chicago, Ill.

evacuating the gas sample valve, closing the stopcock in the vacuum line and then opening the gas-collecting tube stopcock. The aliquot was then introduced into the gas chromatograph in the normal way by means of the gas sample valve. For a complete rumen gas analysis two aliquots were used, one for each column.

Calculations

The volume per cent of each constituent was calculated by a modification of the method described by Johns (8).

Volume per cent hydrogen, oxygen, nitrogen, methane or carbon monoxide

$$= \frac{\frac{A_{SG}}{A_{MS}}\left[\frac{A_x}{K_x}\right]}{CTA} \times 100$$

Volume per cent carbon dioxide or hydrogen sulphide

$$= \frac{\frac{A_y}{K_y}}{CTA} \times 100$$

Where:

A_{MS} = sum of the peak areas on the molecular sieve chromatogram.

A_{SG} = the peak area for hydrogen, oxygen, nitrogen, methane, and carbon monoxide on the silica gel chromatogram.

A_x = peak area for hydrogen (A_H), oxygen (A_O), nitrogen (A_N), methane (A_M), or carbon monoxide (A_{CM}) on the molecular sieve chromatogram.

A_y = peak area for carbon dioxide (A_{CD}) or hydrogen sulphide (A_{HS}) on the silica gel chromatogram.

K_x = relative response factor for hydrogen (K_H), oxygen (K_O), nitrogen (K_N), methane (K_M) or carbon monoxide (K_{CM}).

K_y = relative response factor for carbon dioxide (K_{CD}) or hydrogen sulphide (K_{HS}).

$$CTA = \frac{A_{SG}}{A_{MS}}\left[\frac{A_H}{K_H} + \frac{A_O}{K_O} + \frac{A_N}{K_N} + \frac{A_M}{K_M} + \frac{A_{CM}}{K_{CM}}\right] + \frac{A_{CD}}{K_{CD}} + \frac{A_{HS}}{K_{HS}}$$

Results and Discussion

A variety of column arrangements is possible with the Beckman GC-2 gas chromatograph. Because there was a possibility of obtaining a complete analysis with a single aliquot, some of the arrangements described by Johns (8) and others devised by the authors were tried. However, these

prolonged the elution times and seriously reduced the sensitivity with respect to the minor components. Although the column arrangement described herein required two aliquots for a complete analysis, the advantages of short analysis time, 12 minutes, and high sensitivity outweighed the disadvantage of using two aliquots.

In preliminary experiments it was found that a 6-foot molecular sieve column would separate hydrogen, oxygen, nitrogen, methane, and carbon monoxide over the range of concentrations occurring in rumen gas. The optimum conditions were determined by chromatographing at various pressures, temperatures, and filament currents a gas mixture of the composition: carbon dioxide 67.5, methane 27.5, nitrogen 3, carbon monoxide 0.5, and hydrogen sulphide 0.5 per cent by volume. In each experiment the ratios of the retention times and peak areas were the same for all gases and, therefore, only the values for methane are presented in Table 1. The time between the tail end of the nitrogen peak and the start of the methane peak was important because in this period the operator had to determine when the nitrogen was completely eluted then change the attenuation. This information is given in the last line of Table 1.

The shortest retention time for methane was 1.5 minutes in Experiments 6 and 8. However, the interval between the nitrogen and methane peaks was only 5 seconds, which was not adequate time for the operator to decide when to change attenuation. The 15-second interval in Experiment 7 was adequate, the elution time of 2.2 minutes not unduly long, and the response (peak area) greatest. The conditions of Experiment 7 were the most satisfactory. Under the conditions of this experiment, a 1.5-foot silica gel column was the shortest that would give adequate separation of carbon dioxide. The time for the complete elution of carbon monoxide from the molecular sieve column was 6.5 minutes and for elution of hydrogen sulphide from the silica gel column 5.5 minutes.

Molecular sieve columns separated all the components of rumen gas but under the conditions of this study the retention time for carbon dioxide was

TABLE 1.—DETECTOR CELL RESPONSE IN RELATION TO OPERATING CONDITIONS OF GAS CHROMATOGRAPH, 6-FOOT MOLECULAR SIEVE COLUMN

Experiment no.	1	2	3	4	5	6	7	8
Chromatograph conditions								
Helium pressure, p.s.i.g.	30	40	30	40	30	40	30	40
Filament current, ma.	250	250	350	350	250	250	350	350
Detector response								
CH_4 retention time, min.	3.6	2.5	3.6	2.5	2.2	1.5	2.2	1.5
CH_4 peak area, in.^{2}	3.8	2.5	10.3	7.3	4.0	2.7	12.0	7.9
N_2-CH_4 baseline, sec.*	30	20	25	10	10	5	15	5

* Time recorder pen is on zero between nitrogen and methane peaks.

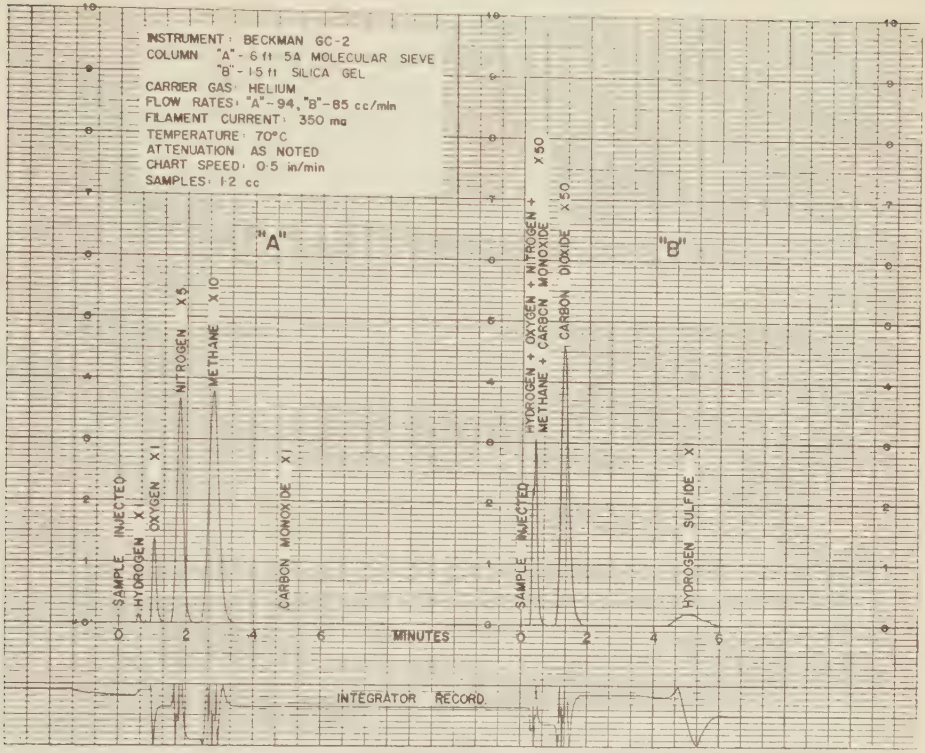

FIGURE 1. Chromatograms for rumen gas of calculated composition: hydrogen 0.59, oxygen 0.38, nitrogen 7.47, methane 25.89, carbon monoxide 0, carbon dioxide 65.48, and hydrogen sulphide 0.19 per cent.

approximately 5.25 hours and for hydrogen sulphide about three times as long. These retention times limited the time which the molecular sieve could be used to approximately 5 hours. In routine analysis of large numbers of samples it was found convenient to use the silica gel column during the first half of the day and to keep a portion of each sample for the second half of the day when the molecular sieve was used. The columns were purged overnight at 220°C. under helium pressure 10 p.s.i.g. and were ready for use the next day. Newly packed columns were purged in the same manner.

The method for calculating the composition is a modification of one described by Johns (8, p. 43). This method was used because it was not necessary to know sample size or to do frequent calibrations.

In the analysis, the aliquots were not under the same pressure, and further, the response of a gas was not the same on the two columns. For the calculation, all peak areas were brought to a common basis, i.e., the peak area the component gas would give on the silica gel column. The molecular sieve column separates hydrogen, oxygen, nitrogen, methane, and carbon monoxide but with the silica gel column a mixture of these gases gives a

single peak (Figure 1). The contribution to the area of this peak by each of the gases in the sample was calculated by multiplying each peak area on the molecular sieve chromatogram by the ratio of the peak area of the mixture with the silica gel column to the sum of the peak areas on the molecular sieve chromatogram, i.e., $(A_{SG}/A_{MS})A_X$. Carbon dioxide and hydrogen sulphide were separated by the silica gel column and their peak areas were obtained directly from the chromatogram. Thus the peak areas of all components were obtained relative to the silica gel column and the volume per cent of each could be calculated from the formula given by Johns.

Typical chromatograms for a rumen gas are presented in Figure 1. Samples containing carbon monoxide have a peak on the molecular sieve chromatogram almost 5 minutes after the sample is injected. The position where a carbon monoxide peak would appear is marked on Figure 1. The relative peak areas were obtained from the integrator record on the lower part of the chart.

It was inherent in the method of calculation that the peak areas of all component gases present had to be known. Over a period of 2 years, during which several hundreds of samples from both bloated and non-bloated cattle on a number of feeds were analysed, the chromatograms afforded no indication of the presence of any component gases other than those determined. No difference has been found between dried aliquots and those analysed as taken. However, the rumen gases must have contained water vapour and vapours of volatile fatty acids and other low boiling rumen constituents. The column packings adsorb water and become inactive. Since inactivation did not take place, it was assumed that the purging procedure desorbed the water. Other materials in the vapour phase would also be adsorbed in the columns. Whether these were removed by the purging, or if they were present in such small quantity that their effect would appear only after long use, was not known. After the columns had been in use for some time the peaks were a little closer together on the chromatograms. This may have been due to inactivation of a portion of the packing, which would give the effect of a shorter column. However, if these substances were removed from the sample by the columns, the analytical results would not be affected because they were independent of the volume injected; the total volume of the components recorded on the chromatograms was the sample size.

Determination of Relative Response Factors

Materials and Methods

The relative response factors used in the calculations were determined with C.P. (chemically pure) grade gases in the unmodified gas chromatograph with instrumental conditions described above for the analyses. All gases except hydrogen were introduced into the chromatograph with a 1.2-millilitre (nominal value) gas sampling valve after thorough flushing. The amounts of contaminant gases were calculated from their peak areas on the

chromatograms. The peak areas for the pure gases were then calculated at 730 millimetres mercury pressure. The peak areas for methane, oxygen, nitrogen, and carbon monoxide were determined with the 6-foot molecular sieve column and those for methane, carbon dioxide, and hydrogen sulphide with the 1.5-foot silica gel column. The relative response factor for each gas was calculated by dividing its corrected peak area by that for methane on the same column, i.e., the response factor for methane was taken as one.

For hydrogen the quantity and method of injection into the chromatograph was somewhat different. A length of rubber hose was attached to the hydrogen cylinder and the valve opened to give a fairly fast gas flow. The needle of a Beckman liquid sampler, set at 50 microlitres, was inserted into the hose near the valve, the sampler flushed with hydrogen several times, then withdrawn and the contents quickly injected into the chromatograph by way of the liquid sample inlet. Methane was handled in the same manner. The relative response factor for hydrogen was calculated from the corrected peak areas.

Results and Discussion

The relative response factors were determined in three separate experiments over the period of a year. Although the columns were repacked and were used in routine analyses of rumen gases, the factors did not differ. Ten to fourteen determinations were made on each gas (Table 2).

The chromatograms obtained with oxygen had only a single peak. Under the conditions used, even minute quantities of hydrogen, nitrogen, methane, carbon monoxide, carbon dioxide or hydrogen sulphide would have been detected. Also the oxides of nitrogen (5, 11) and the rare gases except argon (7) would have produced peaks on the chromatograms. Argon is present in air to the amount of 0.94 per cent and its boiling point is close to that of oxygen (6). On molecular sieve columns argon is not separated from oxygen. From the data of Messner *et al.* (10), it was calculated that the response to argon would be about ten per cent greater than to oxygen. The oxygen had a specification of 99.6 per cent minimum. Therefore, the maximum error in the oxygen response factor would be only 0.04 per cent if argon was the contaminant. In the course of the work, samples of oxygen from several cylinders, including C.P., hospital and industrial grades, were used and all gave the same response factor.

TABLE 2.—RESPONSE FACTORS FOR THE COMPONENTS OF RUMEN GAS RELATIVE TO METHANE

Gas	Mean	Standard deviation
Hydrogen	0.030	0.0038
Oxygen	1.038	0.0047
Nitrogen	1.099	0.0066
Carbon monoxide	1.132	0.0042
Methane	1.000	0.0037
Carbon dioxide	1.380	0.0087
Hydrogen sulphide	1.416	0.0072

The relative response factor for hydrogen could not be determined in the same way as for the other gases because with helium as carrier gas 1-millilitre samples gave both positive and negative deflections on the chromatogram. However, in quantities up to 50 microlitres, hydrogen gives only a positive peak (8). When the carrier gas was helium, the relative response of hydrogen was one-thirtieth of that of the other gases. When nitrogen was used as the carrier, the gas chromatograph was very sensitive to hydrogen but nitrogen itself could not be determined. Furthermore, because the thermal conductivity of nitrogen was near those of the other gases, the chromatograph was relatively insensitive to them. For these reasons helium was used as the carrier in the rumen gas analysis.

In the determination of the relative response factors, the gases were put into the chromatograph at atmospheric pressure and it was necessary, therefore, to correct for differences in pressures of the samples. In this area the mean barometric pressure is approximately 730 millimetres mercury and the peak areas were corrected to this pressure on the assumption that the gases obeyed the perfect gas laws.

The relative response factors are not affected by column temperature, filament current, carrier gas flow rate, column length, column packing particle size or amount, concentration of gas, or thermal sensing element and are independent, therefore, of instrument and operator (8, 10). In this study the factors were determined with approximately 1 millilitre of gas. This raised the question of their reliability for measuring trace amounts of gas. Messner *et al.* (10) found that the relative responses were constant over the range 5 millilitres to 0.1 microlitre. The higher value was five times the sample volume used in the work described in the present paper and the lower value corresponded to a concentration of 0.01 per cent. The properties of relative response factors indicated that it should be possible for other workers to use the method without the necessity of calibrating the instrument.

PRECISION AND SENSITIVITY

Because no methods were known that would approach the method for versatility and sensitivity, and because equipment was not available for preparing gas mixtures of accurately known composition, it was decided to measure the precision of the methods. For this purpose, duplicate samples of rumen gas were taken from each of four cows daily for 5 days. Duplicate analyses were run on each of the 40 samples. The variance components for sampling and analyses were calculated (1, p. 410) for each component gas from the experimental data, except for carbon monoxide which gas was not detected in any of these samples (Table 3).

Variance due to analyses was very low indicating good precision of the instrument and analytical procedures. Variance due to sampling was higher and was attributed to changes occurring in the rumen gas between samplings and was consequent on ingestion of air or differential removal of the gases by absorption.

TABLE 3.—COMPOSITION OF RUMEN GAS AND VARIATION DUE TO SAMPLES AND ANALYSES

Gas	Mean per cent	Variance components	
		Samples	Analyses
Hydrogen	0.18	0.0073	0.0027
Hydrogen sulphide	0.01	0.000007	0.000005
Oxygen	0.56	0.0151	0.0004
Nitrogen	7.00	0.6922	0.1277
Methane	26.76	0.7475	0.0620
Carbon dioxide	65.35	0.9293	0.1170

Peak heights of 0.02 inch on the chromatograms were readily detected and further they were reproducible from aliquot to aliquot. Peaks of this size corresponded to approximately 0.07 per cent for hydrogen and 0.002 per cent for the other gases. It has been shown that the response-concentration curves are linear down to very small quantities (8, 10). It is believed, therefore, that the results are reliable even at these low concentrations.

CONCLUSIONS

Gas-solid chromatography is a convenient method for analysing rumen gas. A complete analysis can be done in less than 15 minutes. Variations in sample volume and the presence of water or other vapours which do not appear on the chromatogram do not influence the results. Frequent sampling is possible because the small sample required reduces the disturbance to rumen contents. An attractive feature of the method is that, with the relative response factors presented herein, analysis of rumen gases can be undertaken directly without having to calibrate the instrument.

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METHOXYL ANALYSIS OF FEEDS AND FECES: A COMPARISON OF METHODS[1, 2]

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ABSTRACT

The Zeisel and hydrolytic methods for the determination of methoxyl were compared both for pure compounds and for forages together with the related feces collected during digestibility trials.

The methods, which were both modified in some respects, showed comparable precision. On pure compounds the hydrolytic method gave results only 1 per cent lower than the Zeisel method but on feeds and feces it gave results 10-15 per cent lower. This discrepancy was shown to be due, in part, to the interference of N- and S-methyl groups.

Highly significant correlations were obtained between the two methods and also between the dry-matter digestibilities and the methoxyl values obtained by either method. Reasons are given why the hydrolytic method is preferred when methoxyl is used as an indicator of digestibility.

Lignin, the least digestible constituent of plant material, has been used as an internal indicator or marker in animal nutrition studies for many years. Since the lignin structure contains firmly attached methoxyl groups a measure of the lignin content can be obtained by determining methoxyl. Thus the methoxyl content of forage and feces, without direct reference to lignin, has been used as an indicator for digestibility by several workers (1, 11, 12).

The Zeisel method, which is generally used for the determination of methoxyl, involves a reaction with hydriodic acid followed by separation and determination of the methyl iodide produced. However, this method has certain disadvantages when used routinely and to avoid these the hydrolytic method of Mathers and Pro (10) was tried. It utilizes a hydrolytic cleavage of methoxyl with sulphuric acid to give methanol. The methanol is separated by distillation and, after oxidation to formaldehyde and reaction with chromotropic acid, is determined colorimetrically.

A comparison of these two methods was carried out using, in addition to pure compounds, grass and legume mixtures together with the related feces collected during digestibility trials.

EXPERIMENTAL PROCEDURES

Zeisel Method

The apparatus was essentially that of Zacherl and Krainick (14) as modified by White (13), except that the funnel above the reaction flask was left off. The procedure was that of Brauns (4) except for the scrubber mixture. Heron *et al.* (6) used 25 per cent sodium acetate as the scrubber solution to reduce the solubility of methyl iodide. It was found that the efficiency of the sodium acetate scrubber could be increased by the addition of a small amount of red phosphorus which traps any iodine carried over from the reaction. In later determinations this scrubber was replaced by a tube containing a layer of anhydrone followed by a layer of ascarite as suggested by Filipovic and Stefanac (5).

[1]Contribution No. 12 of the Analytical Chemistry Research Service, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

[2]Presented in part at the 43rd Annual Conference, Chemical Institute of Canada, Ottawa, Ont., June, 1960.

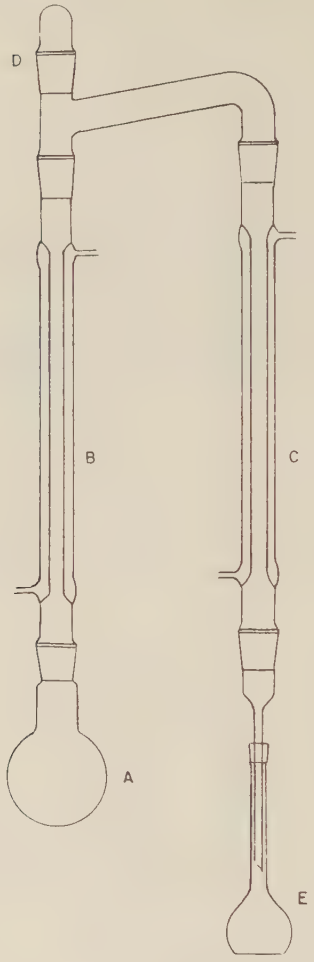

FIGURE 1. Apparatus for methoxyl determination by the hydrolytic method.

Hydrolytic Method

The apparatus is shown in Figure 1 and is an elaboration of that suggested by Mathers and Pro (10). Its advantage is that the flask (A) need not be shifted between the hydrolysis and the distillation. Instead, the cooling water is drained from the first condenser (B) and the methanol solution is distilled through this and condensed in the second (C).

Because a number of changes were introduced the final procedure is described below:

A weighed sample (approximately 1 gram of forage or feces) was placed in the 250-millilitre round bottom reaction flask (A) and concentrated sulphuric acid (20 millilitres) was added through the condenser (C). The mixture was heated over a low flame until fuming commenced and then for a further 5 minutes. The use of a hood is recommended, since sulphur

dioxide is evolved during the reaction. After cooling, distilled water (105 millilitres) was added to the flask by removing the ground glass stopper (D). Distillation from the flask was started after the cooling water was drained from the reflux condenser (C). Heating was resumed and 85-90 millilitres of distillate was collected in a 100-millilitre volumetric flask (E) containing 6 millilitres of 95 per cent ethanol. The contents were made up to the mark with distilled water.

The oxidation and colorimetric determinations were carried out according to the Mathers and Pro procedure but with some modifications. These workers recommended that the chromotropic acid reagent should be made up daily. It was found that by adding a small amount of sodium bisulphite and refrigerating at 36°F. the reagent was stable for at least a week. Our work confirmed Beyer's finding (3) that the heat generated by the addition of the sulphuric acid was sufficient for complete colour development and that a further heating period was unnecessary. Sulphuric acid was used instead of water for the final dilution to avoid a further heating effect. A reduction of the final volume from 50 to 25 millilitres added to the sensitivity of the method.

The final solutions were brought to 25.0°C. in a constant temperature bath and readings were taken on a Klett-Summerson colorimeter using a No. 54 filter. It was found in preliminary experiments that the accuracies obtained were essentially equal using either the Klett-Summerson or a Beckman DU spectrophotometer at 575 millimicrons. However, the strong acid solutions could be transferred much more easily to the cells of the former instrument.

Gas Chromatographic Method

The simultaneous analysis of methoxyl and ethoxyl groups was carried out using a modification of the method of Martin and Vertalier (9). A magnesium perchlorate drying tube and a dry-ice-cooled trap containing 0.2 millilitre of a chloroform-ethylene chloride-methylene chloride mixture (9.5-9.5-1) was connected to the Zeisel apparatus (Figure 2). A 2-millilitre per minute flow of nitrogen was continued for 60 minutes with the reaction flask held at 140°C. The contents of the cold trap were weighed and the alkyl iodides were determined on an aliquot using a Perkin-Elmer 154C vapor fractometer with a 2-metre column of celite containing a silicone 550 liquid phase at 80°C. and hydrogen as the carrier gas.

RESULTS AND DISCUSSION

A Bureau of Standards sample of anisic acid, which theoretically contains 20.4 per cent methoxyl, was analysed by both methods. The averages of six replicates together with the average deviations were 20.3 ± 0.15 per cent for the Zeisel method and 20.1 ± 0.15 per cent for the hydrolytic method. Thus, the latter method gives comparable precision but slightly lower (*ca.* 1 per cent) results.

In Table 1 the two methods are compared on samples of forage and feces. Although the results by the hydrolytic method are lower than those obtained using the Zeisel method by 10 to 15 per cent the highly significant correlation between them indicates that this difference is consistent.

One possible explanation for the higher results by the Zeisel method is that, in addition to methoxyl, other alkoxyl groups are also present in the feeds and feces. In order to test this possibility a method was sought which would simultaneously determine both methoxyl and higher alkoxyls, the most likely possibility being ethoxyl.

The method of Makens, Lothringer and Donia (8) was tried for the microdetermination of methoxyl and ethoxyl. In this method, which is a modified Zeisel determination, the methyl and ethyl iodides are converted to quaternary ammonium iodides by reaction with trimethylamine in nitrobenzene solution. The tetramethylammonium iodide which is formed from the methyl iodide is less soluble in the nitrobenzene solution than is the ethyltrimethylammonium iodide and this solubility difference is said to allow the two alkoxyls to be separated.

TABLE 1.—A COMPARISON OF METHODS IN THE DETERMINATION OF METHOXYL

Description of material	No. samples	Average % methoxyl		Correlation coefficient
		Zeisel	Hydrolytic	
Exp. 408 Forages	17	1.66	1.48	0.957**
Exp. 460 Forages	14	1.70	1.37	0.969**
Total Forages	31	1.68	1.43	0.906**
Exp. 408 Feces	17	1.95	1.70	0.961**
Exp. 460 Feces	42	2.31	2.01	0.991**
Exp. 482 Feces	12	2.76	2.43	0.996**
Total Feces	71	2.30	2.01	0.992**
Total forages and feces	102	2.11	1.83	0.989**
Average variance	102	(0.378)	(0.299)	

** Significant at the 1% level of probability

TABLE 2.—PER CENT RECOVERIES FROM MIXED SAMPLES OF VANILLIN AND p-ETHOXYBENZOIC ACID

Sample number	Methoxyl	Ethoxyl
1	93.9	97.3
2	92.7	94.5
3	92.2	101.6
4	90.2	97.9
5	93.1	100.1
Averages	92.4	98.3

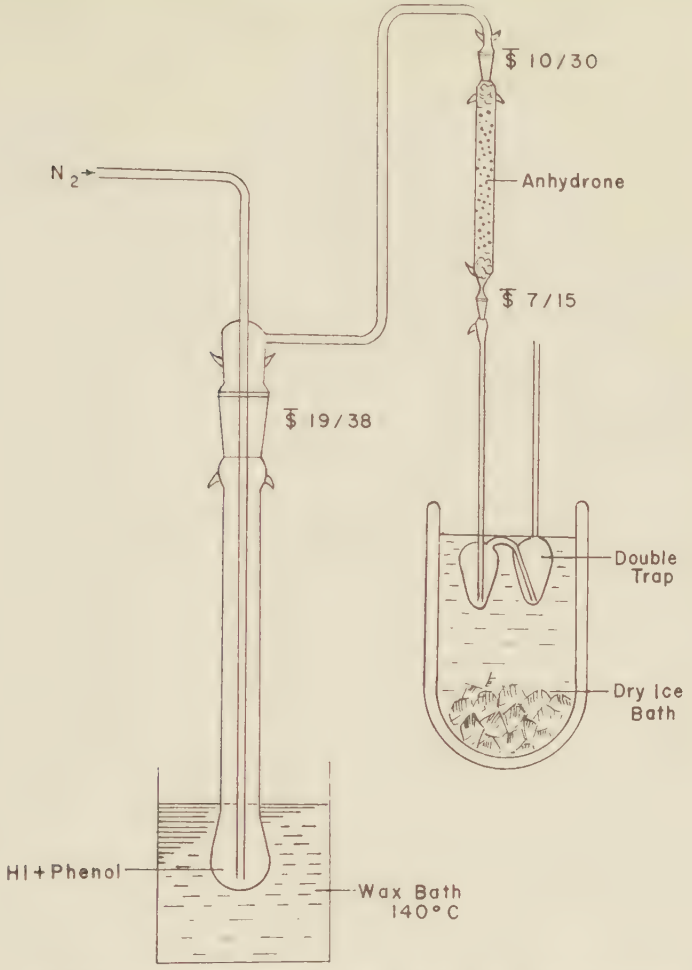

FIGURE 2. Apparatus for alkoxyl determination by the gas chromatographic method.

On investigation we found that the solubility of the tetramethylammonium iodide was markedly affected by the presence of the ethyltrimethylammonium iodide and without knowing, in advance, the concentration of the latter a correction, as suggested by Makens *et al.*, for the solubility of the tetramethyl salt cannot be made. Thus, for the simultaneous determination of the two alkoxyls the method, as it stands, is not suitable.

The gas-phase chromatographic method of Martin and Vertalier (9) was then tried. In their method the initial digestion with hydriodic acid is carried out in a sealed tube. The tube is then opened and placed so that the volatile components can be swept through into a trap containing a chloroform methylene chloride mixture at dry-ice temperature. The contents of the trap are subsequently analysed in a gas-phase chromatograph. The authors claimed, at best, to determine only the molar ratio of methoxyl to ethoxyl.

TABLE 3.—APPARENT METHOXYL CONTENT BY TWO METHODS

Compound	Formula	Per cent methoxyl	
		Zeisel	Hydrolytic
1. Creatinine	$CH_3NC(:NH)NHCOCH_2$	0.01	0.11
2. p-Methylamino-phenol sulphate	$(HOC_6H_4NHCH_3)_2H_2SO_4$	0.11	0.18
3. Sarcosine	CH_3NHCH_2COOH	1.75	0.04
4. N-Methylacetanilide	$CH_3CON(CH_3)C_6H_5$	1.18	0.13
5. DL-Methionine	$CH_3SCH_2CH_2CH(NH_2)COOH$	9.81	1.58
6. Glycyl-DL-methionine	$NH_2CH_2CONHCH(COOH)CH_2CH_2SCH_3$	4.41	0.94
7. 2-Methylthio-4(5)-methylimidazole	$NHC(SCH_3):NC(CH_3):CH$	0.82	0.00

By using a more efficient cold trap (Figure 2) we were able to obtain recoveries on mixed samples of vanillin and p-ethoxybenzoic acid as shown in Table 2.

Using this method on a number of representative samples of feeds and feces we found no trace of ethoxyl and no indication of propoxyl or isopropoxyl groups. Thus, it appears that some other explanation must be sought for the differences found between the hydrolytic and Zeisel methods.

A number of N-methyl and S-methyl compounds were analysed by both the Zeisel and hydrolytic methods and the results are shown in Table 3. It is seen that the N-methyl compounds (1-4) give a small positive reaction with the Zeisel method but little or no reaction with the hydrolytic method. However, the S-methyl compounds, especially methionine, give a distinctly positive reaction by the Zeisel method which is several times larger than that given by the hydrolytic method. Since forage crops are known to contain methionine, at least part of the discrepancy between the two methods can be explained on this basis.

Recovery experiments using the two methods were carried out on both grass and feces using various known amounts of added vanillin. Seven determinations by each of the Zeisel and hydrolytic methods gave average per cent recoveries of 101.2 and 100.5 respectively. The corresponding standard deviations were 2.8 and 2.0.

The methoxyl determination can be used in the evaluation of forages if a sufficiently good correlation between the methoxyl value and digestibility can be found. Regression and correlation coefficients and standard errors of estimate were calculated using a series of feed and feces samples on which dry matter digestibility data were available* and these are shown in Table 4. The regression equations on the totals are as follows:

Forage — Zeisel $y = 94.86\text{-}16.85x$; hydrolytic $y = 104.40\text{-}23.46x$
Feces — Zeisel $y = 85.15\text{-}7.00x$; hydrolytic $y = 85.03\text{-}8.01x$
where y = dry matter digestibility and x = methoxyl value.

*Pigden, W. J., and G. J. Brisson. *Unpublished data.*

TABLE 4.—REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN METHOXYL BY TWO METHODS AND DRY MATTER DIGESTIBILITY

Description of material	Number of samples	Zeisel method			Hydrolytic method		
		Regression coefficient	Standard error of est.	Correlation coefficient	Regression coefficient	Standard error of est.	Correlation coefficient
Exp. 408 Forages	17	−12.817	3.374	−0.591**	−13.205	3.295	−0.616**
Exp. 460 Forages	5	−35.036	1.794	−0.977**	−24.266	2.326	−0.961**
Total Forages	22	−16.846	3.526	−0.694**	−23.460	2.892	−0.807**
Exp. 408 Feces	17	−12.125	2.554	−0.792**	−14.635	2.629	−0.776**
Exp. 460 Feces	15	−11.213	2.074	−0.956**	−12.396	1.854	−0.965**
Exp. 482 Feces	12	−10.980	2.050	−0.977**	−12.335	1.765	−0.983**
Total Feces	44	−6.997	4.673	−0.711**	−8.009	4.598	−0.722**

** Significant at the 1% level of probability

It is seen that, in every case, the correlations are highly significant. A comparison of the two methods using a test proposed by Hotelling (7) and described by Baten (2) indicated no significant difference between the two. Thus, the methoxyl values by either the hydrolytic or Zeisel method would be equally good as indicators of digestibility, especially if the analyses were carried out on the feces.

However, for the following reasons, the hydrolytic method is preferred for the routine determination of methoxyl in forage and feces: (a) A more rugged apparatus is used which can be assembled from standard parts; (b) Less expensive reagents are employed; (c) This allows larger samples to be analysed thus reducing the sampling error; (d) The method is more specific for methoxyl since other alkoxyls are not included and the error due to the presence of N- and S-methyl groups is much smaller.

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AN APPRAISAL OF SELECTION IN THE ROMNELET SHEEP[1]

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ABSTRACT

The amounts of selection for birth weight, weaning weight, post-weaning gain up to about 1 year of age and yearling clean fleece weight in the development of the Romnelet breed of sheep are reported.

The selection differentials for weaning weight only were statistically significant ($P < 0.05$) for both sexes. Analyses of regression of mean differential on time showed an upward trend in the differentials for birth weight and weaning weight, but there was no evidence of significant yearly trend in the differentials for post-weaning traits. Trends in selection pressure were consistent with the reported genetic trends in performance after the initial decline in performance from F1 to F2.

Weaning weight received more attention in selection than any other trait studied, and the most genetic improvement was made in this trait. The large reduction in numbers of animals available for selection from birth to one year of age, due mainly to heavy culling of lambs at weaning, brought about a reduction in selection intensity from 22 per cent to 10 per cent for post-weaning gain and from 42.5 to 22 per cent for clean fleece weight.

INTRODUCTION

Reports on the effectiveness of selection for economic traits in farm animals have generally indicated less response than expected on the basis of the estimated heritabilities of those traits.

Dickerson *et al.* (1) found that performance had declined during the development of 38 lines of swine at seven state experiment stations in spite of positive selection for size of the litter at birth and at weaning and individual weight at weaning and at 154 days of age. The authors pointed out that the effective heritability of parental superiority in phenotype is limited by variation due to environment, dominance and epistasis and by genetic and environmental correlations between components of performance.

Terrill (7) reported that selection differentials for economically important traits in inbred lines of Rambouillet sheep had been nearly all positive, yet body weight and fleece weight declined slightly from 1938 to 1948. There were indications of improvement in weanling index, wool staple length, type score, neck folds, face covering and condition.

Doney (2) found that inbreeding without selection caused a reduction in body size, fertility, grease and clean fleece weight and wrinkle score, but had relatively little influence on percentage yield of clean wool, staple length, fibre diameter and fibre density.

Estimates of genetic trends in performance of the Romnelet sheep during the period of breed development (1935 to 1955) at the Experimental Farm, Manyberries, Alberta, have been reported (6). As a generation scoring device was used to permit intra-year analyses, these trends were not obscured by year-to-year environmental fluctuations. It was shown that

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TABLE 1. — WEIGHTED ANNUAL AND AVERAGE SELECTION DIFFERENTIALS IN POUNDS FOR PERFORMANCE TRAITS IN THE ROMNELET SHEEP

Year of birth	Birth weight			Weaning weight			Post-weaning gain			Clean fleece weight		
	Male	Female	Average	Male	Female	Average	Male	Female	Average	Male	Female	Average
1939	−0.80	−0.12	−0.46									
1940	−0.01	−0.01	−0.01									
1941	0.09	0.12	0.10									
1942	−0.36	0.11	−0.12									
1943	0	0.23	0.12									
1944	1.36	0.26	0.81	5.79	3.43	4.61						
1945	0.02	0.09	0.06	2.68	1.28	1.98						
1946	1.11	0.42	0.76	−1.57	3.44	0.94				0.40	−0.03	0.18
1947	0.49	0.23	0.36	5.21	1.81	3.51				1.26	0	0.63
1948	0.40	0.20	0.30	12.14	1.72	6.93				0.23	−0.02	0.10
1949	−0.29	0.22	−0.03	4.37	1.83	3.10	3.15	0.25	1.70	0.10	0.05	0.08
1950	0.38	0.34	0.36	7.03	3.80	5.42	3.74	0.21	1.98	0.52	0.02	0.27
1951	0.93	0.22	0.57	13.64	2.86	8.25	1.64	−0.64	0.50	0.52	0.05	0.28
1952	2.18	0.60	1.39	23.50	3.33	13.42	5.67	0.47	3.07	0.16	0.10	0.13
1953	0.95	0.29	0.62	25.29	5.05	15 17	4.09	2.07	3.08	0.38	0.25	0.32
Weighted average	0.37	0.20**	0.28	8.06**	2.83**	5.44	3.13	0.51	1.82	1.22	0.16	0.69
										0.45**	0.08	0.26

**Significant ($P < 0.01$)

birth weight, weaning weight, 18-month weight of ewes and yearling clean fleece weight declined sharply from the first-cross generation to F2. There was a slight, further decline in birth weight and clean fleece weight from F2 to F3. No significant change in any of these traits occurred from F3 to F5. From F5 to F7, there was a slight, further decline in birth weight, a slight, non-significant, increase in 18-month weight of ewes and yearling clean fleece weight, and a substantial increase in weaning weight.

The present paper reports the amount of selection for these traits practised in the development of the breed.

MATERIALS AND METHODS

Within the limits of the data available, an appraisal of selection was made for the period of breed development, extending up to the time of the 1955 lamb crop.

The amount of selection practised was estimated by intra-year analysis of variance comparisons of the mean performance of selected animals and all animals expressing the trait that were born in the same year. Separate selection differentials were computed for ewes and rams. In order to avoid errors of inference due to differences in age of dam, type of birth or rearing (single or twin), and age of animal at time of expression of the trait, intra-class analyses were performed on age-adjusted data according to procedures used in the estimation of generation differences (6).

The data were not complete enough for some of the early years to permit the computation of selection differentials for both sexes. Differentials for both sexes were computed for birth weight from 1939 to 1953, for weaning weight from 1944 to 1953, and for yearling clean fleece weight from 1945 to 1953. Data on 18-month weight of rams were not available, but spring weights were available for yearling rams and ewes born in the years 1949 to 1953. Selection differentials were computed for post-weaning gain to about 1 year of age; within each year and sex, the duration of the post-weaning gain period was constant for all animals.

In order to express the selection differentials for each trait in terms that would permit comparison with those for other traits and with potential maxima, the male and female differentials for each year were divided by their standard deviations. Pooled standard deviations for sex-year groups were derived from the sums of squares within age of dam-state of birth or rearing sub-classes, deducting one degree of freedom in each sub-class.

Percentages of potential maximum selection differentials actually applied were computed for the years 1949 to 1953, inclusive, by dividing the selection differentials (in standard deviation units) by the corresponding differentials that would be attainable if percentage of replacements needed was the only limiting factor. The potential maximum selection differentials are the ratios of the ordinates of the normal curve and the corresponding areas under the normal curve representing the various proportions of animals selected. On the average, 26 per cent of the ewes were replaced and 9 rams were used each year during this period.

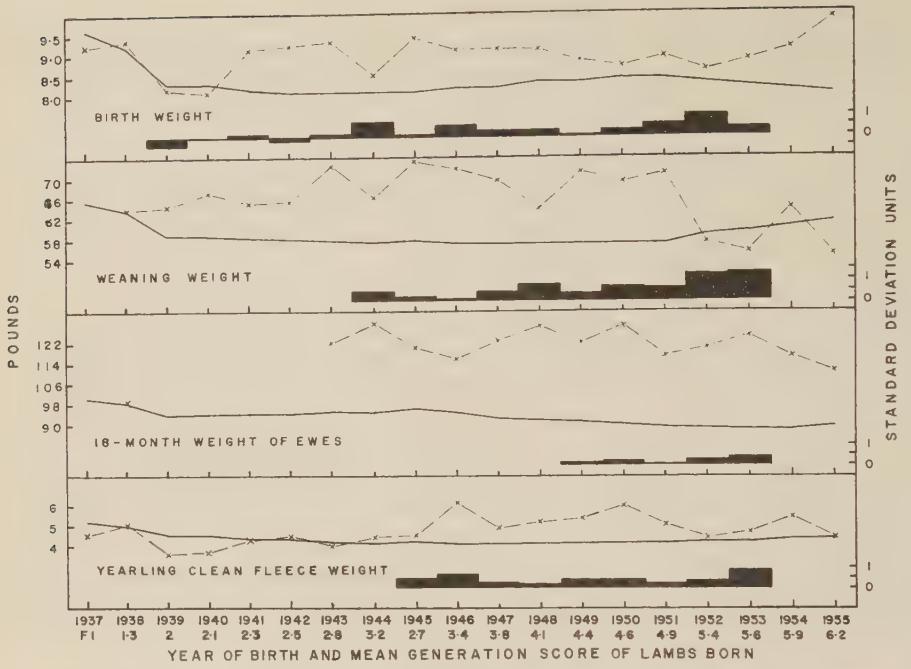

FIGURE 1. Genetic trends in performance (*solid lines*), annual means in performance (*broken lines*) and annual selection differentials in standard deviation units (*histograms*).

RESULTS AND DISCUSSION

The annual selection differentials in pounds are given in Table 1. Differentials in standard deviation units, with the genetic trends and yearly means in performance, are shown graphically in Figure 1. Differentials shown under the curve for 18-month weight of ewes are for post-weaning gain to one year of age. Means in performance of the F1 animals born in 1938 were used as reference points in plotting the genetic trends. The reduction in mean generation score from 1944 to 1945 resulted from the use of 6 Romeldale rams in the 1944 breeding season. A generation score of zero was assigned to the Romeldale and Targhee rams introduced into this project and to all foundation animals.

The differentials for weaning weight only were statistically significant for both sexes. Analyses of regression of mean differential on time showed significant ($P < 0.05$) upward trends in the differentials for birth weight and weaning weight, but there was no evidence of significant yearly trend in the differentials for post-weaning gain and clean fleece weight.

Although there was a linear regression of 0.07 pound per year in the mean birth weight selection differentials, the magnitude of these differentials was not great enough to bring about any improvement in birth weight (6). Birth weight is not important, however, except in so far as it is correlated with survival and post-natal growth rate.

The information available on weaning weights for the years 1939 to 1942 yielded the following intra-class selection differentials: male, 2.57 pounds for 1941; female, 0.16, 0.71, 1.44 and 0.19 pounds for 1939, 1940, 1941 and 1942, respectively.

There was a sharp increase in the weaning weight selection differentials in the later years, due possibly to the fact that horns, wrinkling, excessive face covering and variation in body conformation and fleece characters had been largely eliminated through selection, thereby permitting greater emphasis on performance traits. Consequently, the regression of weaning weight differential on time from 1944 to 1953 was curvilinear. This relationship is described by the equation

$$\hat{Y} = 5.331 - 1.764X + 0.278X^2$$

where $\hat{Y}$ is the selection differential in the year X. The trend in differential was consistent with the reported (6) genetic trend, in which weaning weight increased by 7.8 pounds from F5 to F7.

As the weighted mean selection differential for post-weaning gain was non-significant, it is not surprising that no significant improvement was found for 18-month weight of ewes (6).

Genetic trends in post-weaning gain to 1 year of age and yearling weight were computed by the methods of Peters *et al.* (6) as a further check on this aspect of performance. There were homogeneous, non-significant, intra-year regressions of 0.4 pound and 0.5 pound of post-weaning gain per generation for rams and ewes, respectively. Intra-year regressions of yearling weight on generation were 3.8 pounds and 0.6 pound for rams and ewes, respectively. The regression for rams was significant ($P < 0.01$). There was no heterogeneity of regression between years (1949 to 1955) for either sex.

Since there was no clear evidence of improvement in post-weaning growth rate, it seems likely that the slight upward trend in yearling body weight resulted mainly from the upward trend in weaning weight during this period.

Annual unweighted selection differentials for yearling grease fleece weights of rams and ewes born in the years 1936 to 1944, inclusive, ranged from −0.8 pound to 0.9 pound and averaged zero for both sexes.

Although there was a significant ($P < 0.01$) average selection differential of 0.45 pound in clean fleece weight for rams from 1945 to 1953, this amount of selection pressure was not enough to promote genetic improvement in the absence of significant selection pressure for this trait in ewes.

The birth weight selection differentials computed on an intra-class basis were only 50 per cent as large as those computed without regard for the effects of age of dam or type of birth. Similarly, the weaning weight differentials computed on an intra-class basis were only 64 per cent as large as those computed without regard for age of lamb, age of dam and type of rearing. This observation is in agreement with the previous finding (5) that singles were favoured over twins at selection, and re-emphasizes the need

TABLE 2. — AVERAGE PERCENTAGES OF POTENTIAL MAXIMUM SELECTION DIFFERENTIAL APPLIED FOR PERFORMANCE TRAITS, 1949 TO 1953, INCLUSIVE

Trait	Percentage of potential maximum selection differential applied					
	Based on number of live lambs at birth			Based on number of animals available for selection at the time of expression of the trait		
	Male	Female	Average	Male	Female	Average
Birth weight	23	26	24.5	23	26	24.5
Weaning weight	51	42	46.5	56	47	51.5
Post-weaning gain	13	7	10.0	18	26	22.0
Clean fleece weight	30	14	22.0	40	45	42.5

for consideration of these major environmental influences at the time breeding stock replacements are being selected.

Weaning weight received more attention in selection than any other trait studied; this was true even when consideration was given to numbers of animals available for selection at the time of expression of the trait (Table 2). The large reduction in numbers of animals available for selection from birth to 1 year of age, as the result of heavy culling of lambs at weaning, brought about a reduction in selection intensity from 22 per cent to 10 per cent for post-weaning gain and from 42.5 per cent to 22 per cent for clean fleece weight.

The computed differentials for post-weaning traits may have underestimated the amount of selection pressure on these traits, since a large proportion of the animals born were culled at weaning, and body weight was one of the main criteria for selection at weaning time. The correlated response must have been slight, however, for, although there was a significant upward trend in yearling weight of rams, there was no significant improvement in body weight of ewes or yearling clean fleece weight. MacNaughton (4) found a very low genetic correlation (0.06) between weaning weight and shearling weight in Rambouillet and Canadian Corriedale sheep maintained under range conditions similar to those at Manyberries.

Vesely and Slen (8) have reported heritability estimates of 0.28 for weaning weight, 0.37 for yearling body weight and 0.48 for yearling clean fleece weight in the Romnelet. It appears that there may be more additive genetic variation for these post-weaning traits than for weaning weight remaining in the breed, although it should be possible to make improvement in all of these traits through selection.

As stated formerly (5), inbreeding coefficients of the 1955 lambs ranged from 0 to 26 per cent and averaged 8 per cent. Covariance analysis in the present study showed no significant relationship between degree of inbreeding and birth weight, weaning weight, post-weaning gain to about 12 months of age, grease fleece weight or clean fleece weight of the animals

born in 1955. Nevertheless, inbreeding appeared to have an adverse effect on survival of twin lambs up to weaning age, as the average inbreeding coefficient of twins raised singly and twins that died was significantly ($P < 0.01$) higher than that of twins raised as twins. The weighted means and pooled standard deviations of inbreeding coefficients in the two groups, computed within age of dam and sex, were 8.7 ± 3.6 and 5.8 ± 3.1, respectively.

Multi-trait selection as practised during the development of the Romnelet breed greatly limited the amount of selection pressure that could be applied to any one trait. More than a dozen traits considered to have economic importance received emphasis at selection (5). As stated by Lush (3), when culling simultaneously for n independent and equally important traits, selection for each trait will be $1/\sqrt{n}$ as intense as if all the efforts of selection could have been concentrated on that trait alone. Thus, under these conditions, selection for 12 traits simultaneously would result in 29 per cent of the potential maximum selection differential for each trait.

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SOME OBSERVATIONS ON THE RELATIONSHIP BETWEEN VITAMIN B_{12} AND REPRODUCTION IN SWINE[1]

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ABSTRACT

Six female swine were maintained during one or more gestations on a vitamin B_{12}-deficient diet. Two that had been fed the deficient diet for more than 2 years prior to their first gestation aborted. Four that received the deficient diet for 7 months prior to their first gestation farrowed a total of 8 litters containing 68 live piglets; 27 died within 3 days. All piglets fed a synthetic milk diet, deficient in vitamin B_{12}, died within 14 days.

Five adult females fed the deficient diet supplemented with 400 μg. of vitamin B_{12} per animal per day farrowed a total of 8 litters containing 83 live piglets; none died within 3 days. Of 14 piglets fed a vitamin B_{12}-deficient synthetic milk diet, 2 died within 14 days.

The different dietary vitamin B_{12} intakes of the adults influenced the serum vitamin B_{12} levels of the adults and of their offspring.

Piglets fed vitamin B_{12} gained more, but feed conversion was not influenced by the vitamin B_{12} dietary intake of the piglets or by the intake of their dams during gestation.

It is concluded that vitamin B_{12} influences swine reproduction and neonatal survival.

INTRODUCTION

During the past decade a number of reports have dealt with the influence of vitamin B_{12} on the reproduction of various species of animals, including swine. Most, but not all of the evidence indicates that vitamin B_{12} does influence reproduction, and that the dietary intake of vitamin B_{12} might be important for swine maintained under practical husbandry conditions (4).

The work reported here was carried out over a period of several years to clarify various aspects of the relationship between vitamin B_{12}, reproduction and neonatal performance.

EXPERIMENTAL

Adults

Eleven female swine, approximately 7 months old, received a vitamin B_{12}-deficient, corn-soybean meal diet (7), with or without a 400 μg. supplement of vitamin B_{12} per animal per day, for different periods of time prior to their first mating and during subsequent gestation periods according to the following plan:

	Basal diet						Basal diet plus vitamin B_{12}				
Sow no.	1	2	3	4	5	6	7	8	9	10	11
Age at 1st mating (months)	36	36	15	15	21	15	36	36	15	15	15
No. gestations studied	1	1	2	3	2	1	1	1	3	2	1

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The basal diet was fed twice daily. It contained less than 1 μg. of vitamin B_{12} per pound, but not less than N.R.C. (9) recommended allowances of other nutrients. Animals 7 and 8 received no vitamin B_{12} supplement for the first 18 months and variable amounts until 2 months before conception. A supplement of 400 μg. of vitamin B_{12} daily was fed to animals 7 and 8 from 34 months of age and to animals 9-11 from 8 months of age. Animals 7-11 are designated hereafter as "treated" animals and animals 1-6 as "control" animals.

Animals were kept individually in pens on raised wooden floors covered with shavings. They were exercised twice weekly for 30 minutes on a mechanical exerciser that forced the pigs to walk at the rate of 3 m.p.h.

Blood samples were taken usually at 2-week intervals from the major blood-vessels in the region of the thoracic inlet. The 10 weeks before conception was considered the pre-gestation collection period. Reported values (Table 1) represent the mean serum vitamin B_{12} levels for the specified periods.

Piglets

Stillbirths, live births and neonatal deaths up to 3 days of age were recorded. Some of the piglets were removed from their dams on the third morning after birth. They were assigned to oral vitamin B_{12} supplementation tests as follows:

Piglet test 1

	Treated sows No.			
	7		8	
Oral vitamin B_{12} (μg./piglet/day)	0	20	0	20
Number of piglets	3	3	3	3
Observation period (days)	26	26	43	43

Piglet test 2

	Control sows					Treated sows			
	3		4		5	9		10	
Litter No.	1	2	1	2	1	1	2	1	2
Vitamin B_{12}/kg. dry matter intake (μg.)	No. piglets at each dosage level								
0	2	2	2	2	1	2	2	2	2
20	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	1
80	1	1	1	1	1	1	1	1	1

Piglets in test 1 were grouped together in metal cages according to treatments and dams. Those in test 2 were kept in individual cages for either 53 or 56 days. All test piglets were fed six times daily between 7 a.m. and 8 p.m. a vitamin B_{12}-deficient, synthetic milk diet similar to the medium fat diet of Cunningham and Brisson (2). Modifications of the diet included the use of vitamin-free casein, adjustment of the solids content from 10 to 15 per cent, and deletion of the vitamin B_{12} supplement. A solution of crystalline vitamin B_{12} was administered *per os* to the treated piglets in test 1, and it was added to the milk fed to the treated piglets in test 2. Feed efficiencies were calculated as kilograms synthetic milk dry matter consumed/kilogram body weight gained.

Of the piglets that remained with the control dams, one-half received intramuscular injections of vitamin B_{12} soon after birth.

Blood samples were obtained by the method described for sows or by cardiac puncture.

Vitamin B_{12} Assay

Vitamin B_{12} was assayed with *Euglena gracilis var. bacillaris* (5).

Data were analysed by common statistical methods.

RESULTS AND DISCUSSION

Blood serum vitamin B_{12} levels of control sows were distinctly lower than those of treated sows (Table 1). This was true both prior to and during the different gestations.

TABLE 1. — BLOOD SERUM VITAMIN B_{12} LEVELS OF SOWS

Sow No.	Gestation	Serum vitamin B_{12} ($\mu\mu$g./ml.)				
		Pre-gestation	Days of gestation			
			0–28	29–56	57–84	85–42
		Control sows				
1	1	151	117	89	113	155
2	1	123	143	97	114	137
3	1	64	11	56	10	98
	2	—	95	—	132	84
4	1	136	83	70	37	84
	2	—	90	—	159	129
5	1	77	115	—	127	134
	2	—	—	—	117	101
6	1	<20	—	—	—	<20
		Treated sows				
7	1	437	224	254	257	290
8	1	274	195	162	228	—
9	1	544	564	315	362	438
	2	—	592	438	374	405
10	1	470	531	383	383	551
	2	—	431	403	417	443
11	1	280	—	—	—	326

TABLE 2. — REPRODUCTIVE PERFORMANCE OF SOWS

Sow No.	Gestation	Farrowing history			Remarks
		Total born	Still-births	Neonatal deaths	
		Control sows			
3	1	12	0	1	
	2	12	2	3	
4	1	11	0	0	
	2	11	1	4	
	3	9	1	8	
5	1	11	2	3	
	2	9	3	6	
6	1	6	4	2	2 were deformed; 1 was born a day later.
Total		81	13	27	
		Treated sows			
7	1	13	7	0	Deaths were caused by anoxia resulting from prolonged delivery.
8	1	13	0	0	
9	1	15	2	0	
	2	15	1	0	
	3	10	2	0	The stillbirths were born a day later.
10	1	14	3	0	
	2	10	0	0	
11	1	11	3	0	
Total		101	18	0	

Sows 1 and 2, fed the deficient diet for the longest period prior to mating, aborted near term. Post-mortem examinations of their genital tracts revealed no evidence of infection.

The maternal dietary vitamin B_{12} status markedly influenced the survival of piglets born alive (Table 2). Clinical observations of successive litters born by basal-fed sows revealed progressively weaker newborn piglets. This is indicated only partially in Table 2; the effect was observed in offspring of sows maintained under practical conditions (6). Similar observations have been reported for rats (8) and mice (1). Piglets left with control dams survived only if they were given intramuscular injections of vitamin B_{12}.

Table 3 extends and corroborates the preceding observations on the effect of the different maternal and neonatal regimens on neonatal survival.

The original weight gain data revealed no apparent differences in the weight gains between piglets treated with 20, 40, or 80 μg. of vitamin B_{12} per kilogram of dry matter intake. Therefore the results for these treatments were grouped together. Variance analysis of mean litter weights of

TABLE 3. — EFFECT OF MATERNAL AND PIGLET VITAMIN B_{12} DIETARY INTAKES ON PIGLET SURVIVAL AND 8-WEEK WEIGHT GAINS (PIGLET TEST 2)

Vitamin B_{12} in piglet diet (μg./kg. D.M.)	Control sows			Treated sows		
	Piglet weight gain (kg.)		Survival ratio[1]	Piglet weight gain (kg.)		Survival ratio[1]
	Mean	S. E.		Mean	S. E.	
0	—	—	0/9	15.7	1.1	7/8
20	19.5	0.5	3/5	20.6	1.6	4/4
40	17.7	3.5	3/5	21.7	1.4	3/4
80	19.5	1.4	3/5	19.4	1.3	3/4

[1]Number of survivors/number initially allocated

treated piglets, either unweighted or weighted according to the number of piglets studied per litter, did not reveal any difference in piglet growth due to the maternal dietary vitamin B_{12} intake during gestation. Apparently, the deficiency acquired *in utero* was reversible in these treated piglets as far as weight gains were an indication, but this requires additional observations. It agrees with observations in mice (1) and rats (3, 8).

Comparison between final body weights of vitamin B_{12}-treated and untreated piglets born of treated sows were made on a within-litter basis, after adjustment for initial body weights. The mean difference for each litter was weighted inversely according to its variance, and the mean of these weighted differences was significant (P almost 0.001). Weight differences between treated and untreated piglets occurred in different litters at widely different times after allocation (2 to 8 weeks). The wide between-litter time differences may be part of the explanation for the widely different estimates of the vitamin B_{12} requirement of baby pigs reported in the literature.

In all groups there was a direct relationship between body weight gained and the amount of milk consumed. Average feed conversion efficiency for the different groups ranged between 7.2 and 7.7 kilograms dry matter consumed per kilogram body weight gained. Differences between groups were not apparent ($P > 0.05$).

The vitamin B_{12} dietary intake of dams influenced the blood serum vitamin B_{12} levels of piglets. Serum levels of three 3-day-old piglets born by control sows 3 and 4 ranged from 40 to 44 $\mu\mu$g./ml.; levels of four similar-aged piglets born by treated sows 9 and 10 ranged from 156 to 366 $\mu\mu$g./ml. Table 4 shows the effect of the neonatal dietary treatment of piglets born by treated sows 9 and 10 on serum levels at various intervals during the feeding trials. It also includes information from piglets born by treated sows 7 and 8. At each time interval the serum levels of untreated piglets were lower than those of treated littermates. Despite the different

TABLE 4. — EFFECT OF VITAMIN B_{12} SUPPLEMENTATION OF SYNTHETIC MILK DIET ON PIGLET BLOOD SERUM VITAMIN B_{12}($\mu\mu$g./ml.)[1]

Sow No.	Piglet age (weeks)	Piglet vitamin B_{12} supplement[2]			
		0	20	40	80
7	4	20-57 (3)[3]	239-724 (2)		
8	4	156-240 (2)	460-542 (2)		
	6	14-130 (2)	381-524 (2)		
9	8	162 (1)	265 (1)	231 (1)	258 (1)
10	8	134 (1)	260 (1)	258 (1)	191 (1)

[1]Data from piglet tests 1 and 2
[2]As μg./day for progeny of sows 7 and 8 and μg./kg. dry matter intake for progeny of sows 9 and 10
[3]Number of piglets in parentheses

TABLE 5. — BODY WEIGHT GAINS (KG.) OF PIGLETS BORN OF TREATED SOWS 7 AND 8 (PIGLET TEST 2)

Sow	Vitamin B_{12} dose (μg./day)	
	0	20
7	3.0	3.0
	3.2	3.7
	5.6	—
Mean	3.9	3.4
8	8.7	18.0
	6.8	16.3
Mean	7.8	17.2

4-week serum levels of treated and untreatd piglets born by sow 7, differences in weight gains were not apparent at that time (Table 5). This agrees with our belief that serum levels are a more sensitive criterion of vitamin B_{12} status in the growing animal than weight gains. Weight gain differences between treated and untreated offspring born by sow 8 occurred as early as 16 days after birth. The 16-day body weights of piglets born by treated sow 8 were compared with similar data from the litters born by treated sows 9 and 10. When the mean litter differences in the 16-day body weights were adjusted for the initial weights, the mean difference between the treated and untreated piglets born by sow 8 was significant ($P < 0.05$). Blood samples were taken from 4-week-old piglets born by sow 8. Differences in serum vitamin B_{12} levels between treated and untreated piglets were apparent and the levels of the same untreated piglets declined further during the following 2 weeks.

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AN APPRAISAL OF CERTAIN SOURCES OF ENVIRONMENTAL VARIATION IN THE PRODUCTIVITY OF YORKSHIRE SOWS[1]

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ABSTRACT

Analysis of the records of 616 Yorkshire litters farrowed over a 20-year period in the University of Alberta swine herd indicated that litter performance was not associated with differences in weight changes of the sow during gestation or with gestation length. Gestation weight change was negatively correlated with breeding weight and lactation weight changes. Age of sow, breeding weight and litter order were highly correlated and were mutually dependent in their effect on litter performance. Least squares estimates of year, season, breeding weight of sow, and weight change during lactation effects were obtained for several performance traits of the litter. Year differences were large but erratic for all traits. Seasonal differences appeared to be important only for litter size with fall litters averaging 0.78 more pigs at weaning than spring litters. Weight of sow at breeding exerted an important influence on litter performance. Weight change during lactation appeared to be a function of litter size.

Study of the relative influence of age and weight of gilts at breeding on first litter size at birth and weaning indicated that breeding weight exerted the greater influence.

It is concluded that comparisons between litters in the same herd and born within the same year and season must provide for statistical control of variation in weight of dam at breeding. If inter-year comparisons are desired additional statistical control is essential.

INTRODUCTION

The wide variation in sow productivity as measured by the performance of her litter constitutes a major problem for the breeder who is attempting, through selection, to improve his swine herd, or for the nutritionist conducting reproduction and lactation experiments. This variation consists of two major components, environmental and genetic variability.

Environmental variation impedes the rate of genetic progress by causing errors in judgement of genotypic merit. Two approaches may be employed in coping with this variation. The first is to eliminate such variation, in so far as this is possible, through the use of environmental and biological control. This is rarely practical or feasible and the alternative of statistical control must be considered.

The variation attributable to non-genetic factors provides an indication of the importance of environmental control and indicates the specific type of program that will most likely be effective in achieving over-all progress. Contributions in this field of swine breeding have been forthcoming from numerous sources; of particular significance are reports by Korkman (3), and Lush and Molln (5), each of which involved studies on over 5,000 pigs. The only Canadian study in this field, that of Sinclair and Syrotuck (6) in 1928, examined the influence of the age of sow on birth and weaning numbers and weights of pigs.

The work reported herein represents an evaluation of several environmental factors with which sow productivity is associated.

[1]Contribution from the Department of Animal Science, University of Alberta, Edmonton, Alberta, and from the Research Branch, Canada Department of Agriculture, Lacombe, Alberta.

[2]Adapted from a thesis submitted in partial fulfilment of the requirements for an M.Sc. degree. The senior author was holder of a National Research Council of Canada bursary.

TABLE 1.—UNCORRECTED YEAR AND SEASON MEANS FOR SIX CLASSIFICATIONS OF SOWS AND FIVE PERFORMANCE TRAITS OF THE LITTER

Year and season	n	Means for sow traits						Means for litter performance				
		Breeding weight	Farrowing age	Litter order	Gestation length	Weight changes		Litter size			Litter weights	
						Gestation	Lactation	Total born	Born alive	Weaned	Birth	Weaning
		lb.	mo.		days	lb.	lb.				lb.	lb.
1934	22	443.3	30.6	3.0	115.3	48.5	−10.9	10.6	9.6	6.9	24.5	226.8
1935	24	378.8	23.1	2.5	115.6	60.3	−19.3	11.2	10.5	7.9	27.0	250.2
1936	34	405.8	28.6	3.6	115.4	41.9	−19.8	11.6	11.0	8.4	30.1	242.0
1937	27	434.4	31.0	3.9	114.6	45.5	−3.6	11.9	10.7	8.2	29.1	255.8
1938	30	407.5	29.5	3.7	114.4	71.4	−20.9	12.4	11.4	9.4	34.6	304.3
1939	32	444.2	31.4	4.1	115.4	75.5	−33.6	11.9	11.2	8.7	32.0	253.5
1940	32	416.6	28.4	3.7	115.5	71.9	−30.8	11.3	10.6	8.6	31.4	259.1
1941	23	443.3	34.8	4.5	115.0	96.1	−40.0	12.3	11.2	8.8	32.4	285.2
1942	39	423.4	29.2	3.6	114.8	77.3	−36.0	10.4	9.5	7.8	28.8	252.2
1943	31	409.9	30.2	3.6	114.6	78.4	−39.5	11.1	10.4	8.0	30.9	247.6
1944	45	429.4	31.8	3.9	114.5	85.5	−56.0	10.9	10.4	7.4	29.8	204.7
1945	41	433.4	35.1	4.2	114.8	84.7	−42.4	10.7	10.2	7.0	28.7	186.9
1946	40	421.4	33.0	3.9	114.5	70.2	−4.5	10.3	9.9	6.9	27.1	200.0
1947	30	371.3	28.0	3.1	114.9	71.8	−16.1	11.5	11.1	7.3	30.6	183.2
1949	27	357.2	27.5	2.1	114.6	60.2	−30.1	11.6	10.7	7.8	31.4	199.7
1950	13	382.3	28.9	3.7	115.5	140.0	−61.9	10.8	9.5	7.8	25.4	228.2
1951	41	374.2	24.6	2.9	115.4	82.0	−61.7	10.2	9.7	7.3	25.3	193.3
1952	34	416.8	31.0	3.4	113.8	54.8	−43.4	11.1	10.1	7.7	25.9	240.6
1953	51	405.3	28.6	2.9	114.0	64.7	−26.3	10.4	9.4	7.6	25.8	234.5
Spring	355	414.3	30.1	3.5	114.9	73.9	−36.2	11.1	10.2	7.6	29.2	218.8
Fall	261	405.9	29.5	3.5	114.7	65.8	−25.6	11.1	10.4	8.1	28.5	248.4

DESCRIPTION OF DATA AND ANALYTICAL PROCEDURE

Data for this study were obtained from records accumulated for the swine herd, Department of Animal Science, University of Alberta, Edmonton, during the 20-year period 1934-53. The litters were all of registered Yorkshire parentage and were produced by an outcrossing system comparable to the mating program in common use by breeders of Yorkshire swine during the period.

Sow productivity was measured by the litter characteristics of total number born, number born alive, number weaned, litter birth weight of living pigs and litter weaning weight. All litters were weaned at 56 days of age.

The environmental influences considered were year and season of birth; and the sow traits were breeding weight, age at farrowing, gestation number, length of gestation, and weight changes during gestation and lactation.

All records for which data were incomplete, or records where either the sow or the litter up to weaning age were involved in nutritional trials, were rejected. The data remaining after application of these restrictions included 616 litters out of 214 dams and sired by 40 boars.

Data rejected included all litters of size zero at weaning. Since these data were distributed at random across the sow population it was considered unlikely that this procedure would introduce bias.

The uncorrected data for average performance of the sows and their litters by year and season are shown in Table 1. Rather large annual fluctuations were evident for all traits but there was no evidence of a directional trend over the period under investigation.

Because of the non-orthogonal nature of these data, analysis by the method of least squares was indicated. It was clear, however, that the several sources of environmental variation attributable to sow differences could not be considered mutually independent. For example, weight at breeding, age at farrowing and gestation number provide different measures

TABLE 2.—CORRELATIONS BETWEEN SIX MAJOR CLASSIFICATIONS OF THE SOW

Sow class	Farrowing age	Litter order	Gestation weight change	Lactation weight change	Gestation length
Breeding weight	.79	.76	−.38	−.06	−.02
Farrowing age		.96	−.24	−.11	−.03
Litter order			−.08	−.01	−.02
Gestation weight change				−.42	−.01
Lactation weight change					.05

of the same trait. These interrelationships are illustrated by the correlations of Table 2. The correlations of breeding weight with age at farrowing and gestation number of .79 and .76 respectively and that of .96 between age at farrowing and gestation number are comparable to the correlations reported by Kozeluka (4) and Stewart (7). The correlation of —.38 between breeding weight and weight change during gestation is in general agreement with results obtained by Zeller *et al.* (8). Gestation length was essentially unrelated to all of the other traits measured. With the exception of the correlation of —.42 between gestation weight change and lactation weight change, these traits showed no important association with the other traits. This information provided a basis for designation of the independent variables to be used in analysis.

Year, season, weight at breeding and lactation weight change were the four independent variables included in the least squares solution. Gestation length was excluded because of its low variability and because other workers (1, 6) have not found it to influence litter performance appreciably. Weight change during gestation was excluded because of its apparent association with breeding weight and weight change during lactation. Breeding age and gestation number, both functions of time, were discarded. Sire effects were included in corrections for years and seasons.

TABLE 3.—ESTIMATES OF THE ENVIRONMENTAL EFFECTS OF YEAR (EXPRESSED AS DEVIATION FROM y_1) AND SEASON ON FIVE PERFORMANCE TRAITS OF THE LITTER

	Total pigs born	Pigs born alive	Pigs weaned	Litter birth weight	Litter weaning weight
				lb.	lb.
y_2	1.07	0.95	0.64	3.27	10.26
y_3	1.26	1.39	1.20	5.77	7.29
y_4	1.32	1.02	1.22	4.79	28.10
y_5	2.12	1.77	2.18	10.67	68.15
y_6	1.31	1.41	1.34	6.87	12.01
y_7	0.75	0.65	1.10	6.42	15.47
y_8	1.45	1.17	1.38	6.84	42.24
y_9	0.12	0.07	0.58	4.83	15.33
y_{10}	0.38	0.63	0.51	5.85	5.68
y_{11}	0.06	0.12	−0.46	3.38	−45.89
y_{12}	0.05	0.18	0.34	3.37	−47.85
y_{13}	0.21	0.64	0.23	3.94	−10.67
y_{14}	1.76	1.98	0.26	8.05	−38.19
y_{15}	1.66	1.33	0.78	7.53	−20.02
y_{17}	0.31	−0.75	−0.41	0.22	−37.19
y_{19}	0.43	0.24	0.21	0.64	−0.17
y_{20}	0.15	−0.21	0.30	1.77	−3.21
y_1 mean	10.6	9.6	6.9	24.5	226.8
$s_2 - s_1$	0.22	0.41	0.78	0.28	35.62
s_1 mean	11.1	10.2	7.6	29.2	218.8

The theory of least squares has been discussed by Kempthorne (2) and examples of its use may be found in many published papers. Standard errors of estimates were obtained by the method of matrix inversion. The sub-classes for which least squares estimates were obtained are shown below.

YEAR 1 (y_1), 1934; YEAR 2 (y_2), 1935; ---- YEAR 20 (y_{20}), 1953.
SEASON 1 (s_1) SPRING (JANUARY-JUNE); SEASON 2 (s_2) FALL (JULY-DECEMBER)

Breeding weight (lb.)				Lactation weight change (lb.)			
Sub-class	n	Range	Mean	Sub-class	n	Range	Mean
b_1	57	177–250	232	$1a_1$	74	−189 to −91	−117
b_2	51	251–290	268	$1a_2$	60	−90 to −71	−79
b_3	39	291–330	314	$1a_3$	87	−70 to −51	−61
b_4	67	331–370	354	$1a_4$	82	−50 to −31	−41
b_5	79	371–410	392	$1a_5$	93	−30 to −11	−21
b_6	91	411–450	432	$1a_6$	100	−10 to +9	−2
b_7	87	451–490	471	$1a_7$	56	10 to 29	21
b_8	70	491–530	511	$1a_8$	64	30 to 195	54
b_9	75	531–661	574				

RESULTS AND DISCUSSION

Estimates of Environmental Parameters

The least square constants are presented as the difference from sub-class one in the case of years (Table 3), and as the difference between all sub-classes in the case of breeding weight (Table 4) and lactation weight change (Table 5). The advantage of this classification is that trends throughout years as a whole, and important differences as well as trends related to breeding weight and weight change during lactation, are more clearly illustrated.

Years – Year differences in litter performance were of sufficient magnitude to invalidate comparisons between years. There was a lower level of performance for weaning numbers and weights during the latter half of the period considered. The tendency for weaned litter size and average pig weights at weaning to fall simultaneously is contrary to the trends observed by others from studies on the relationships among litter mates. It would, therefore, appear that both livability and growth suffered a general decline.

The higher average pig weights observed in the last 2 years probably reflect the effect of antibiotic feed supplements which came into use at that time in the University of Alberta's swine creep feed rations.

Season – The average number of pigs weaned and the total litter weights of fall litters exceeded those of spring litters by 0.78 pigs and 35.6 pounds respectively. The increase in litter weight probably reflects little more than the effect of numbers since the average weights of fall pigs exceeded those of spring pigs by only 1.7 pounds.

TABLE 4.—ESTIMATES OF THE ENVIRONMENTAL EFFECT OF BREEDING WEIGHT ON FIVE LITTER PERFORMANCE TRAITS[1]

Litter trait	Mean code	Breeding weight								
		232 b_1	263 b_2	314 b_3	354 b_4	392 b_5	432 b_6	471 b_7	511 b_8	574 b_9
Total pigs born	b_1	8.61	.94*	1.30*	2.10*	2.51*	2.83*	3.51*	3.29*	2.97*
	b_2		9.67	.36	1.16*	1.57*	1.89*	2.57*	2.35*	2.03*
	b_3			10.05	.80*	1.21*	1.53*	2.21*	1.99*	1.68*
	b_4				11.16	.41	.73	1.41*	1.19*	.88*
	b_5					11.24	.32	1.00*	.78*	.47
	b_6						11.53	.68	.46	.14
	b_7							12.17	−.22	−.54
	b_8								12.09	−.31
	b_9									11.65
Pigs born alive	b_1	8.35	1.09*	1.32*	1.84*	2.21*	2.35*	2.81*	2.44*	1.95*
	b_2		9.39	.22	.75*	1.12*	1.26*	1.72*	1.35*	.86*
	b_3			9.96	.52*	.90*	1.04*	1.49*	1.13*	.63
	b_4				10.54	.37	.51	.97*	.60	.11
	b_5					10.62	.14	.60	.23	−.26
	b_6						10.71	.46	.09	−.40
	b_7							11.17	−.37	−.86*
	b_8								10.90	−.49
	b_9									10.31
Pigs weaned	b_1	6.51	.85*	1.03*	1.18*	1.16*	.75*	1.26*	1.24*	.23
	b_2		7.86	.17	.32	.30	−.10	.41	−.39	−.62
	b_3			7.95	.15	.13	−.27	.24	.22	−.79*
	b_4				8.45	−.02	−.42	.09	.07	−.94*
	b_5					8.18	−.40	.11	.09	.92*
	b_6						7.58	.51	.49	−.52
	b_7							8.21	−.02	−1.03*
	b_8								8.31	−1.01*
	b_9									7.19
Litter birth weight, lb.	b_1	21.61	1.50	5.38*	7.16*	7.35*	7.47*	9.00*	8.72*	7.97*
	b_2		23.96	3.88*	5.66*	5.85*	5.97*	7.50*	7.22*	6.47*
	b_3			27.31	1.78	1.97*	2.09	3.62*	3.34*	2.59*
	b_4				30.75	.19	.31	1.84*	1.56	.81
	b_5					29.53	.12	1.65	1.37	.62
	b_6						29.76	1.53	1.25	.50
	b_7							31.30	−.28	−1.03
	b_8								31.41	−.75
	b_9									30.35
Litter weaning weight, lb.	b_1	168.40	26.50*	36.95*	57.16*	50.24*	46.20*	55.46*	52.42*	33.65*
	b_2		223.14	10.45	30.55*	19.70	23.74	28.95*	25.95*	7.06
	b_3			221.54	20.21	13.29	9.25	18.50	15.47	−3.39
	b_4				256.13	−6.92	−10.96	−1.70	−4.74	−23.60*
	b_5					242.68	−4.04	5.21	2.18	−16.67
	b_6						229.67	9.26	6.23	−12.63
	b_7							244.45	−3.03	−21.89*
	b_8								247.57	−18.86
	b_9									227.67

[1]The leading diagonal gives the uncorrected mean of each sub-class. Corrected means of sub-classes were used to obtain environmental effects. Environmental effects are shown as the difference between row and column sub-classes, e.g. ($b_2 - b_1 = .94$), ($b_3 - b_1 = 1.30$).

*An asterisk is placed after each constant that is equal to or greater than twice its standard error.

TABLE 5.—ESTIMATES OF THE ENVIRONMENTAL RELATIONSHIP BETWEEN WEIGHT CHANGES OF THE SOW DURING LACTATION AND TWO PERFORMANCE TRAITS OF THE LITTER[1]

Litter trait	Mean / Code	−117 la_1	−79 la_2	−61 la_3	−41 la_4	−21 la_5	−2 la_6	21 la_7	54 la_8
Pigs weaned	la_1	8.77	−.21	−.91*	−1.09*	−1.37*	−1.79*	−1.97*	−3.22*
	la_2		8.85	−.69*	−.87*	−1.16*	−1.58*	−1.76*	−3.01*
	la_3			8.30	−.18	−.46	−.88*	−1.07*	−2.31*
	la_4				8.15	−.29	−.70*	−.89*	−2.13*
	la_5					7.65	−.42	−.60*	−1.85*
	la_6						7.48	−.18	−1.43*
	la_7							7.29	−1.25*
	la_8								5.97
Litter weaning weight, lb.	la_1	262.92	−20.96	−34.41*	−41.93*	−50.44*	−64.09*	−70.69*	−107.00*
	la_2		255.92	−13.45	−20.97*	−29.47*	−43.12*	−49.12*	−86.03*
	la_3			250.16	−7.52	−16.02	−29.68*	−36.27*	−72.59*
	la_4				239.77	−8.50	−22.16*	−28.76*	−65.07*
	la_5					221.52	−13.65	−20.25*	−56.56*
	la_6						220.60	−6.60	−42.91*
	la_7							217.71	−36.31*
	la_8								178.67

[1]The leading diagonal gives the uncorrected mean of each sub-class. Corrected means of sub-classes were used to obtain environmental effects. Environmental effects are shown as the difference between row and column sub-classes, e.g. ($la_2 - la_1 = -.21$).
*An asterisk is placed after each constant that is equal to or greater than twice its standard error.

Breeding weight — The averages given in Table 4 (leading diagonal) indicate that litter size at birth increased steadily to a maximum at a mean breeding weight of 471 pounds. Average number of pigs living at birth also increased but at a slower rate. Thus as weight of sow at breeding increased from 232 pounds to 574 pounds, the number of stillborn pigs increased from 0.26 to 1.34 pigs. Litter size at weaning increased to a maximum at b_4 (354 pounds) but the greatest change occurred between b_1 and b_2.

It is apparent that an increase in breeding weight was beneficial in terms of total litter size but this benefit was opposed by an increased pre- and post-natal mortality. As a consequence, weaned litter size remained relatively constant throughout the major part of the life of the sow.

The least squares estimates show that differences in breeding weight contributed significantly to variation in total litter size at birth up to a mean breeding weight of 354 pounds, and in numbers born alive up to a mean breeding weight of 314 pounds. In numbers weaned the differences were significant only for the extreme breeding weight classes of 232 and 574 pounds.

The trend in total litter weight at birth appears to be primarily a function of number of pigs born alive. Weaned litter weight, however, reflects the combined effect of average litter size and average pig weight at weaning. Thus litters from sows of classes b_4, b_7 and b_8 were of essentially the same size but were significantly heavier at weaning than litters from sows of class b_2.

These trends in litter performance are basically similar to those reported by other workers (for example 3, 5) for age of sow or gestation number. The minor differences observed may well be attributed to breed or environmental differences rather than to the difference in analytical method.

Lactation weight change — Litter size and weight at weaning were inversely related to weight change during lactation (Table 5). This was not unexpected; weight changes during lactation reflect in large measure the demands of the litter during the nursing period and such demands will be directly related to litter size. It will be noted, however, that average pig weight at weaning remained relatively constant across the range la_1 to la_8. This is contrary to the report of Zeller *et al.* (8), who reported a positive relationship between average weaning weight and weight loss during lactation.

Gilt Litter Size in Relation to Age and Weight at Breeding

The data of the study included 100 litters farrowed by gilts 11, 12 or 13 months of age. These litters were divided within age into two breeding weight classes of equal number consisting of the heaviest and lightest individuals respectively and average litter size for each class and sub-class was computed. These data are given in Table 6.

The litters were then reclassified into three breeding weight groups consisting of the lightest, middle, and heaviest one-third respectively. Each group was then sub-divided into classes on the basis of age at farrowing. Litter sizes calculated for each class and sub-class are also presented in Table 6.

TABLE 6.—THE INFLUENCE OF AGE AT FARROWING AND BREEDING WEIGHT ON LITTER NUMBERS FROM GILTS

Major class	Major class average	Minor class average	No. of litters	Total no. born	No. born alive	No. weaned	Total no. born	No. born alive	No. weaned
Age	11 mo.		28	9.17	9.03	7.13			
		W_1	14				8.29	8.29	5.86
		W_2	14				9.93	9.64	8.21
	12 mo.		52	8.86	8.61	6.77			
		W_1	26				8.62	8.27	6.35
		W_2	26				9.12	8.96	7.19
	13 mo.		20	9.20	8.80	6.95			
		W_1	10				8.50	8.30	6.30
		W_2	10				9.62	9.30	7.60
Breeding weight	W_1		33	8.36	8.18	6.09			
		11 mo.	11				8.73	8.73	6.00
		12 mo.	17				8.41	8.18	6.29
		13 mo.	5				7.40	7.00	5.60
	W_2		33	8.85	8.71	7.09			
		11 mo.	9				8.50	8.40	7.20
		12 mo.	20				8.75	8.55	7.00
		13 mo.	4				10.25	10.25	7.25
	W_3		34	10.09	9.47	7.53			
		11 mo.	8				10.06	10.25	8.62
		12 mo.	15				9.53	9.27	7.00
		13 mo.	11				9.64	9.18	7.45

Litter size in terms of total number born, number born alive, and number weaned showed no consistent patterns when calculated by age or age within weight class. However, there was a consistent and marked increase in litter size as weight or weight within age of gilt increased. These results indicate that weight of gilt at breeding exerts a greater influence on litter performance than does age. Because of the limited numbers involved this trend cannot be considered conclusive. It is, however, in agreement with the results reported by Stewart (7) and suggests that sexual maturity is associated more closely with weight than age.

Application

This study has evaluated the relationship of year and season of birth, breeding weight of the dam and her weight loss during lactation with the performance of the litter. The results have shown litter performance to be conditioned by or related to each of these four factors. These observations have a direct application particularly in nutritional and breeding experiments which deal with productivity of the sow.

Yearly differences for all traits were shown to be erratic and large. Seasonal differences were unimportant except for size of litter at weaning with fall litters weaning on the average 0.78 more pigs than spring litters. From these results it is concluded that direct comparisons between litters may be made only for those born within the same year and preferably the

same season. If inter-season or inter-year comparisons are desired, statistical corrections must be made to the data.

Weight change of the sow during lactation was closely associated with litter performance. However, the importance of lactation weight change as a measure of productivity is not clear since size of litter itself may be a factor conditioning weight loss during lactation.

Breeding weight of the sow contributed materially to variation in litter numbers and weights at birth and weaning, indicating that corrections are necessary to provide unbiased comparisons between sows of different breeding weights. Correction factors for adjustment of data are given in Table 4. For example, to provide an unbiased comparison of litters from sows in classes b_1 and b_2 an adjustment of 0.94 pigs is necessary for total litter size at birth, of 1.09 for litter size living at birth, and 0.85 for weaned litter size. Similar correction factors could be derived to allow comparison of litters within other herds.

Application of correction factors must be tempered by recognition of the possible genetic interrelationship between weight of sow at a given age and the performance of her litter. This study has considered weight of sow at breeding as an environmental character, while actually it is conditioned in part by the genetic constitution of the sow. However, it is not considered that the genetic component of breeding weight will alter appreciably the interpretation of results.

This study has also indicated the importance of weight as contrasted with age relative to litter size from gilts. While it is reasonable to expect that there may be an optimum age for first breeding of gilts, the evidence obtained here would indicate that weight is likely to impose the more serious limitations on litter performance. It would appear highly desirable that gilts attain a specified minimum weight before breeding is permitted.

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DIGESTIBILITY AND FEEDLOT STUDIES ON RAPESEED SCREENINGS FOR FEEDER LAMBS

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ABSTRACT

Screenings from combine-harvested rapeseed were ground and incorporated as 50 per cent of a pelleted feed containing 36.5 per cent ground oat hulls, 4 per cent dehydrated alfalfa meal, 7 per cent cane molasses, 0.5 per cent urea, 1.0 per cent dicalcium phosphate and 0.5 per cent salt. These pellets composed 0, 33, 67 and 100 per cent of the total concentrate allowance fed with hay to four lots of 20 feeder lambs during a 67-day test. The hay allowance was limited to 1.5 pound/head/day. Grain and pellets were full-fed twice daily. In a parallel digestibility study pellets were substituted for concentrate (mixed wheat, oats and barley) at the following levels: 0, 16.7, 33.3, 50 and 100 per cent and with the hay allowance held constant at 50 per cent of the ration.

The pelleted mixture was found to contain 51 per cent T.D.N. and 10.6 per cent D.C.P. (dry matter basis) and when included as one-third of the concentrate fed, the lambs gained 0.47 pound/day. Increasing levels of screenings pellets beyond one-third of the concentrate portion of the ration resulted in decreasing gains proportional to the decline in percentage T.D.N. in the entire ration. Tests for isothiocyanate and thiooxazolidone revealed only traces of these toxic factors.

Rapeseed screenings contain 8 to 11 per cent crude protein, about 57 per cent T.D.N. (10 per cent moisture basis), and appear to be satisfactory as an ingredient in ruminant rations.

INTRODUCTION

With the increasing importance of rapeseed production in Western Canada the efficient utilization of rapeseed screenings as a feed for livestock presents a problem. It is important that the feeding value of this by-product be investigated, particularly in view of the known presence of toxic factors in rapeseed oil meal (1).

Rapeseed screenings are composed of varying proportions of hull or seed pod fragments, immature or small seeds, some weed seeds and other extraneous material retained with the rapeseed during combine harvesting. Preliminary analytical information revealed protein contents approximating those of oats or barley, about 25 per cent crude fibre and a relatively high fat content of 15 to 20 per cent.

This report contains the results of digestibility trials and a feedlot test with fattening lambs in which rapeseed screenings were incorporated into a pelleted feed and substituted at various levels for whole grain in the ration.

MATERIALS AND METHODS

The evaluation of rapeseed screenings was conducted in two phases: a feedlot test involving 80 feeder lambs and a concurrent digestibility study with 15 similar lambs fed the same rations as used in the feedlot trial plus additional combinations of ration components so as to permit calculation of digestibility coefficients for each major feeding stuff in the ration in terms of energy and protein.

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TABLE 1.—INGREDIENT FORMULA FOR RAPESEED SCREENINGS PELLETS

Ingredient	Per cent
Rapeseed screenings	50.0
Oat hulls	36.5
Alfalfa meal, dehydrated	4.0
Molasses, cane	7.0
Urea—262	0.5
Dicalcium phosphate	1.0
Salt, plain	0.5

Ration Components

(a) *The rapeseed screenings* were ground in a hammer mill and employed as one-half of a mixed formula (Table 1). This mixture contained ingredients designed to augment the supplies of vitamin A (carotene), protein, calcium, phosphorus and salt, and was finally made into 3/16-inch pellets.

(b) The *grain mixture* was composed of a mixture of high quality whole grains: barley, oats and wheat in approximately the ratio 4:3:1.

(c) The *hay* used was a well-cured mixture of about equal parts legume and grass and comprising sweet clover, alfalfa, brome grass and western rye grass. It was fed coarsely chopped.

Both water and mineral mixture (containing salt, iodine, cobalt, calcium and phosphorus) were available *ad libitum* to the lambs.

The Digestibility Experiment

These studies involved conventional digestion trials with 10 days' fecal collection preceded by a 14-day preliminary period. For the first 10 days of the preliminary period five lambs were group-fed the ration to be tested, then for the last 4 days four of the lambs exhibiting the best appetites were confined to individual metabolism cages. During this time the twice-daily feedings were standardized at intake levels slightly below those prevailing previously while the lambs were in groups.

Feces were collected twice daily. The evening collection was placed in a polyethylene bag containing 10 millilitres of saturated boric acid solution and stored in a refrigerator. The following morning's collection was added to that of the previous evening and a 1/10 aliquot was saved and composited until a representative sample of 10 days' feces was obtained. Finally the samples were dried at 105°C., ground and re-mixed for analyses.

TABLE 2.—FORMULATION OF RATIONS USED IN THE DIGESTIBILITY TRIALS

Period and ration no.	Hay %	Grain %	Pellets %
1	100	—	—
2	50	50	—
3	50	33.3	16.7
4	50	16.7	33.3
5	50	—	50
6	—	—	100

TABLE 3.—COMPOSITION AND DIGESTIBILITY COEFFICIENTS OF RATIONS FED IN DIGESTION TRIALS. ALL RESULTS EXPRESSED ON A *DRY MATTER* BASIS

Ration fed	Gross energy	Crude protein	Digestibility of			T.D.N.*	D.C.P.
			Dry matter	Crude protein	Gross energy		
	Cal./gm.	%	%	%	%	%	%
Hay, mixed grass-legume	4.31	11.4	59	71	58	57	8.1
Grain, barley + oats + wheat, 4:3:1	4.53	13.6	*82***	*79*	*81*	*84*	*10.7*
Pellets, rapeseed screenings	4.62	14.7	50	72	49	51	10.6
Hay + grain, 1:1	4.42	12.5	71	75	70	70	9.4
Hay + grain + pellets, 3:2:1	4.47	12.7	68	76	66	67	9.7
Hay + grain + pellets, 3:1:2	4.45	12.9	62	74	63	64	9.5
Hay + pellets, 1:1	4.46	13.1	57	70	58	59	9.2

* Calculated on the basis of 2000 digestible Calories/lb. T.D.N.
** Based on by-difference estimates of digestibility since this ration was not fed alone

Feed and feces samples were analysed for total protein (N x 6.25) and for gross energy (Cal./gm.). T.D.N. was calculated by assuming 2000 Cal./lb. T.D.N. (5). The rapeseed screenings pellets were analysed for isothiocyanate and thiooxazolidone by the methods described by Wetter (6, 7).

The sequence of rations tested in the digestibility studies is shown in Table 2.

Limitations of facilities for accommodating sheep during the preliminary periods prevented the adoption of a design that would have maximized the statistical control on the experimental variables. The plan adopted involved prior assumptions that the time factor between periods and the variability due to individual animals were unimportant relative to the differences between digestibility coefficients obtained for the various rations.

The Feedlot Experiment

Four groups of 20 wether lambs from the University flock, randomly allotted within weight and breed classes, were penned immediately after weaning in mid-September, 1959, and assigned to the experimental rations. The treatments were (a) control: hay and grain, (b) hay plus a mixture of ⅓ pellets: ⅔ grain, (c) hay plus ⅔ pellets: ⅓ grain and (d) hay plus pellets only. Hay intakes were limited to a maximum of 1.5 pounds per head daily and the grain or pellets portions of the ration were fed twice daily. The duration of the test was 67 days.

RESULTS AND DISCUSSION

The Digestibility Experiment

By means of chemical and digestibility tests it was shown that (Table 3) the rapeseed screenings pellets contained 14.7 per cent crude protein, which was 72 per cent digestible, thus yielding 10.6 per cent digestible crude protein (D.C.P.) on a *dry matter* basis. The energy fraction was 49 per cent digestible and the T.D.N. content, assuming 2000 Calories per pound of T.D.N. (5), calculated to 51 per cent. As a consequence of this relatively low T.D.N. value the gradual replacement of grain by pellets resulted in the T.D.N. content of the ration dry matter decreasing from about 70 per cent for hay plus grain to 59 per cent for hay and pellets.

The T.D.N. content of the rapeseed screenings *per se*, on a 10 per cent moisture basis, was estimated by attributing values of 32.3, 47.1 and 52.4 per cent T.D.N. to oat hulls, dehydrated alfalfa meal and cane molasses, respectively, as reported by Schneider (4) for sheep and was found to be about 57 per cent. Chemical analyses* on several samples of screenings revealed crude protein contents ranging from 8 to 11 per cent; about 18 per cent crude fat and 25 per cent crude fibre. Chemical tests made on the pellets showed only traces of isothiocyanate and thiooxazolidone to be present thus indicating that the feeding value of rapeseed screenings would be primarily dependent on their digestible nutrient contents and acceptability by the animal.

*Olsen, G., Federated Co-operatives Ltd., Saskatoon, Sask. *Private communication.*

Of interest relative to the design of the digestibility experiment, the differences between rations (or periods) proved to be highly significant ($P<.01$; F test) and the coefficient of variability for the experiment was 4.7 per cent, thus indicating a high degree of uniformity between animals.

The Feedlot Experiment

The results of the feeding test (Table 4) show average daily gains ranging from 0.34 to 0.47 pounds, the highest gains being obtained on a ration containing 33 per cent screenings pellets. This group gained significantly faster (2) than any of the other three groups. Increasing the level of screenings in the ration beyond 33 per cent of the concentrate portion of the ration resulted in declining T.D.N. contents and in declining daily intakes of T.D.N. per lamb, thus supporting the postulation that the over-all feeding value is determined largely by digestible energy or T.D.N. content.

The failure of the grain-fed control lambs to gain as rapidly as those fed one-third screenings pellets may have been due to the unexpectedly high T.D.N. content of the grain (Table 3). Adjustment of 75 per cent T.D.N. (dry matter basis) to a 10 per cent moisture (air-dry) basis yields a value of 67-68 per cent for the control ration which exceeds the 61-63 per cent level recommended by Pope *et al.* (3) and this in turn reflects a T.D.N. level for the grain portion well in excess of the expected value based on published data (4). It is suggested that much of the difference between actual and estimated values was due to the use of Rodney oats which, being a variety of exceptionally low hull content and relatively high fat content, and comprising over one-third of the grain mixture, may have appreciably augmented the digestible energy value.

Considering the possible effects of Rodney oats, together with examination of the bi-weekly records of feed consumption, it is concluded that

TABLE 4.—AVERAGE LAMB PERFORMANCE AND FEED UTILIZATION RESULTS FROM FEEDLOT TRIAL

Criteria of feed and growth	Percentage screenings pellets used in grain portion of ration (%)			
	0	33	67	100
Growth responses				
Initial weight, lb.	70.0	70.2	70.4	68.9
Final weight, lb.	79.9	102.2	96.6	91.5
Daily gain, lb.	0.40	0.47	0.39	0.34
Feed intakes, per day				
Hay, lb.	1.05	1.09	1.09	1.13
Grain mixture, lb.	2.34	1.66	.90	—
Pellets, lb.	—	.83	1.84	2.82
Nutrient intakes and composition data				
T.D.N. intake, lb./day	2.53	2.43	2.31	2.09
% T.D.N. in total ration, dry matter	75	68	60	53
T.D.N./lb. gain, lb.	6.3	5.2	5.9	6.1
% D.C.P. in total ration (dry matter basis)	9.9	9.9	9.9	9.8

the lower gains of the control lambs were related to difficulty in keeping them 'on feed'. The lambs appeared to find grain more palatable than pellets and consequently it was difficult to avoid over-feeding with subsequent periods of underfeeding.

It is concluded that rapeseed screenings may be used successfully in ruminant rations. They should be regarded as a roughage in view of a T.D.N. content in the range of good quality hay and because of the relatively high crude fibre content.

It was not possible to assess palatability of the screenings *per se* in these studies because they were incorporated in a pellet containing several strongly flavoured ingredients and furthermore the pellets exhibited some tendency to crumble, a factor which in itself apparently reduces palatability to sheep.

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EFFECTS OF PORCINE IMMUNE GLOBULIN ADMINISTRATION ON THE SURVIVAL AND SERUM PROTEIN COMPOSITION OF COLOSTRUM-DEPRIVED PIGS REARED IN A NON-ISOLATED ENVIRONMENT

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ABSTRACT

Five experiments involving 90 newborn colostrum-deprived pigs were conducted in an attempt to develop a method of rearing applicable in a non-isolated environment. Immune globulins, prepared by ammonium sulphate fractionation of porcine serum, and comprised of a mixture of approximately 75 per cent γ-globulin and 25 per cent β-globulin, were administered orally or parenterally in varying amounts and for varying periods of time. In two experiments porcine albumin, in serum or in a semi-purified solution, was provided in addition to the immune globulins. The distribution of serum proteins in the pigs was studied from birth to 12 weeks of age by paper strip electrophoresis.

Parenteral administration of immune globulins did not provide an effective passive immunity, nor did oral administration for 1 day post-partum. A marked improvement in survival occurred when oral administration was continued for 5 days, and it was further found that this treatment provided apparently complete protection against infection during the 5-day period of administration. Albumin appeared to further improve survival.

These results, together with the relatively poor survival obtained with positive control pigs (nursed 24 hours) suggested a continuing need for a supply of immune globulins in the lumen of the intestinal tract. Presumably these globulins were active as coproantibodies.

The percentage survival in pigs weighing 3 pounds or more at birth was substantially higher than in smaller pigs. Mortality in these experiments was usually attributable to colibacillosis.

Serum immune globulin levels at 2 days of age in the artificially reared pigs were elevated in proportion to the amount of globulins given. The effect of albumin was to create a serum protein pattern resembling that of suckled pigs. A marked decline in γ-globulin levels from 2 days to approximately 6 weeks was observed.

INTRODUCTION

Newborn pigs, in common with the young of several other species, lack antibodies and are unable to synthesize them for several weeks post-partum (9, 16, 20, 21, 24 and others). Disease resistance during the neonatal period is thus dependent on colostral antibodies, secreted by the sow in large quantities following parturition and absorbed by the piglet, apparently intact, from the small intestine (6, 7, 9, 16, 20).

Ingestion of colostrum during the first 24 hours results in a rapid and striking increase in the more slowly migrating electrophoretic globulin fractions and a comparable increase in antibody titres (2, 16, 20). Absorption of γ-globulin appears to be seriously impaired after 24 hours (24, 25), although some absorption has been reported to occur as late as 72 hours after birth (17).

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Success in rearing colostrum-deprived pigs has, because of the essential nature of colostral antibodies, been largely dependent on the use of elaborate isolation units and specialized techniques (26, 27, 28). Pigs so reared have been employed experimentally (11, 12) and used to establish breeding herds free of atrophic rhinitis and virus pig pneumonia (29). Attempts to raise colostrum-deprived pigs in a non-isolated environment have generally met with little success (2, 3, and others) although Pond *et al.* (19) reported good survival in pigs when antibiotics and porcine γ-globulin were administered during the first 24 hours.

A series of experiments was begun in 1958 at the University of Saskatchewan in an effort to develop a method of rearing colostrum-deprived pigs in a non-isolated environment. The primary objective of the research was development of a technique permitting study of neonatal nutrient requirements under practical conditions. A secondary objective was a method of rearing which would avoid exposure to sow-borne disease, a technique which might prove of particular interest in connection with the production of specific pathogen-free pigs. The general approach has been the provision of passive immunity by oral or parenteral administration of porcine immune globulins.

Results of the initial experiments* indicated that parenteral administration of immune globulins did not provide an effective passive immunity, despite appreciable elevation of serum immune globulin levels. Mortality in almost all cases was attributed to colibacillosis. This condition appeared initially as an intestinal infection which, considered in relation to the demonstrated presence of coproantibodies in human intestinal diseases (1, 4, 10), suggested that a possible explanation for the apparent discrepancy between survival and serum immune globulin levels was the route of administration of the globulins. The artificially-reared animals apparently lacked adequate means of coping with infections of the intestinal tract.

Reported herein are five experiments designed to study oral versus parenteral administration of immune globulins, to compare globulins obtained from the University swine herd with those obtained from abattoir serum and to evaluate possible benefits of orally administered porcine albumin.

MATERIALS AND METHODS

Pigs were taken from the sow immediately after birth and blood samples were obtained from the umbilical cord whenever possible. Otherwise, and for all subsequent bleedings, samples were drawn from the anterior vena cava (5). Pigs were ear-tagged and randomly allotted to treatments. Initial feedings and treatments were administered within 3 hours of birth. Positive control pigs were returned to the sow for 24 hours after which they were fed and managed identically to those artificially reared.

Pigs were individually penned in a well ventilated room maintained between 80° and 90°F. Sanitation practices at farrowing and during the experiments were deliberately kept comparable to those followed in the swine barns.

Unpublished

TABLE 1.—DIET FORMULAS

Milk replacer		Pre-starter		Starter	
Homogenized milk	1000 ml.	Skimmilk powder	190.0 lb.	Ground wheat	506.0 lb.
Egg yolks	1	Whey powder	65.0 lb.	Oat middlings	242.0 lb.
Trace mineral solution[1]	5.0 ml.	Fishmeal	135.0 lb.	Soybean oilmeal	150.0 lb.
Thiamine	3.3 mg.	Linseed oilmeal	100.0 lb.	Fish meal	50.0 lb.
Riboflavin	3.3 mg.	Ground wheat	200.0 lb.	Iodized salt	5.0 lb.
Calcium pantothenate	4.4 mg.	Oat middlings	50.0 lb.	Ground limestone	5.0 lb.
Nicotinic acid	13.2 mg.	Lard	50.0 lb.	Brewers' dried yeast	20.0 lb.
Pyridoxine	2.2 mg.	Wheat germ meal	82.4 lb.	Dehydrated alfalfa meal	20.0 lb.
Vitamin B_{12}	13.2 mcg.	Cane sugar	80.0 lb.	Zinc sulphate	0.26 lb.
Ascorbic acid	40.0 mg.	Brewers' dried yeast	30.0 lb.	Vitamin A and D premix	1.50 lb.
Vitamin A	3300 I.U.	Iodized salt	5.0 lb.	(1500 A — 300 D per gm.)	
Vitamin D	1100 I.U.	Dicalcium phosphate	10.0 lb.	Penicillin	6.2 gm.
Procaine penicillin G	22.0 mg.	Trace minerals[2]	0.57 lb.	Streptomycin	18.8 gm.
Streptomycin	33.0 mg.	Vitamin B_{12} supplement[3]	1.00 lb.	Terramycin	25.0 gm.
		Vitamin A and D premix (1500 A — 300 D per gm.)	1.50 lb.		
		Penicillin	12.4 gm.		
		Streptomycin	37.6 gm.		
		Terramycin	50.0 gm.		

[1] 49.8 gm. $FeSO_4.7H_2O$; 3.9 gm. $CuSO_4.5H_2O$; 3.6 gm. $MnCl_2.4H_2O$; 0.26 gm. KI; 4 ml. conc. HCl; distilled water to 1000 ml.
[2] 100 gm. $FeSO_4.7H_2O$; 10 gm. $CuSO_4.5H_2O$; 150 gm. $ZnSO_4.7H_2O$.
[3] Courtesy, Merck, Sharpe and Dohme of Canada, Ltd., Montreal, Que., Each pound of supplement contained 9 mg. of vitamin B_{12}.

A sow milk replacer (Table 1) was fed in nursing bottles. In Experiments 1, 2 and 3 four feedings of 30 millilitres each were given during the first 24 hours. Thereafter three feedings per day were given, and the quantity of milk replacer was gradually increased to a maximum of 300 millilitres per feeding. In Experiments 4 and 5 immune globulins, albumin or saline were mixed with approximately 15 millilitres of milk replacer to a total volume of 25 millilitres and fed at 2-hour intervals during the first 48 hours. The total volume was increased to 50 millilitres (including approximately 40 millilitres of milk replacer) and the interval between feedings was extended to 4 hours during the next 72 hours, after which three feedings per day were given and the amount of milk replacer gradually increased to 300 millilitres per feeding.

Pre-starter was offered *ad libitum* after the pigs reached 2 weeks of age. Pigs were weaned at 15 pounds, fed pre-starter ration and allowed water *ad libitum* until they weighed 25 pounds, at which time they were switched to a starter ration. The latter was fed until the termination of the experiments at 12 weeks of age. Feed consumption data were not recorded. The formulas of the three diets are shown in Table 1. The milk replacer was a modification of the formula of Young and Underdahl (26). The protein content of the milk replacer, pre-starter, and starter were 3.5 per cent, 28.1 per cent and 22.5 per cent respectively.

Pigs were weighed at birth and at 1, 2, 4, 6, 8, 10 and 12 weeks of age. Blood samples were obtained at the same ages and at 2 days. Electrophoretic separations and total protein determinations were carried out in duplicate on all samples.

Immune globulins were prepared from blood collected from mature sows and 200-pound market pigs, from the University herd, when they were slaughtered at a local abattoir. Fibrinogen was removed by carefully stirring the blood and collecting the fibrin as it formed. Immune globulins, composed of a mixture of approximately 75 per cent γ-globulin and 25 per cent β-globulin were precipitated from the serum by addition of ammonium sulphate to 40 per cent saturation. Gamma-globulin was quantitatively removed at this level of saturation and it was assumed that antibodies migrating as β-globulins would be present as well. The precipitates were dissolved in and dialyzed against 0.85 per cent sodium chloride, shell frozen in a bath of methanol and dry ice and stored at −15°C. The immune globulin solutions as administered ranged from 8 to 11 per cent protein.

The porcine albumin used in Experiment 5 was prepared from the supernatant fraction after precipitation of immune globulins. The level of saturation of ammonium sulphate was increased to 60 per cent and the resulting precipitate of β- and α-globulins was discarded. Albumin was precipitated by further addition of ammonium sulphate to 80 per cent saturation.

A Spinco Model R paper electrophoresis system, with constant current power regulation and Veronal buffer at pH 8.6, 0.075 ionic strength, was employed for electrophoretic separations. Samples of 0.008 millilitre were applied in duplicate on 3.0 x 30.6 centimetre strips of Schleicher and Schuell 2043A paper. Separations were of 16 hours' duration at room temperature.

Strips were stained in a methanol solution of bromphenol blue dye and rinsed in three changes of 5 per cent acetic acid. Evaluation was by elution of the bound dye in 0.5 per cent sodium carbonate, the dye bound by each segment being determined from percentage transmission readings at 590 millimicrons in a Bausch and Lomb "Spectronic 20" colorimeter[1]. These readings gave a measure of the relative percentage of each component in the sample.

No α_1 component was detected in most instances and it was assumed that this component had migrated with albumin. The β- and γ-globulin bands were difficult to separate in some cases, particularly in blood of 2-day-old nursed pigs.

Total serum protein was determined by the method of Lowry *et al.* (15), employing Folin-Ciocalteau phenol reagent. Standards were prepared from a "Protein Standard Solution"[2] of crystalline bovine albumin. Standards and samples were read at 500 millimicrons in a Bausch and Lomb "Spectronic 20" colorimeter. The amount of each serum protein component was determined by multiplying its relative percentage by the total serum protein value.

Post-mortem examinations were conducted on all pigs that died during the experimental periods. Bacteriological examinations of heart, blood, lung, liver and, in some cases, intestinal contents were carried out.

Statistical analyses of data were conducted according to standard methods (22). The 5 per cent probability level was accepted as indicating a significant difference. Duncan's multiple range test (8) was employed for comparisons of means.

Experiment 1 was designed to compare (a) oral with parenteral administration of immune globulins and (b) globulins, precipitated by ammonium sulphate, with equal amounts administered as unfractionated porcine serum. Dosage levels were based on the estimated absorption of colostral immune globulins by suckled pigs during the first 24 hours.

Experiment 2 was initiated to further evaluate the effects of combined parenteral plus oral immune globulin administration, and to determine whether the antibiotic tetracycline, administered parenterally, would improve survival.

Experiment 3 constituted a further attempt to determine whether or not an effective passive immunity could be established by parenteral administration of immune globulins. The level studied in this trial was considerably higher than in the previously-mentioned unpublished experiments, in which parenteral administration had proved ineffective in terms of survival.

The objective of Experiment 4 was to study the effect on survival of continuing the oral administration of immune globulins for a period of 5 days post-partum. The initial dosages of immune globulins were reduced to 15 grams from the 20 grams used in earlier experiments to minimize the problem of distension in the gastro-intestinal tract.

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[1]Procedural details were as given in Spinco Technical Bull. TB 6052A.
[2]Armour Pharmaceutical Co., Chicago, Ill.

TABLE 2.—EXPERIMENTAL TREATMENTS

Expt. No.	Lot No. and Treatment						Mode of administration
	1	2	3	4	5	6	
1	Negative control, physiological saline (6)[1]	1.5 gm.[2] immune globulins by intraperitoneal injection (6)	20 gm. immune globulins orally (6)	1.5 gm. injected + 20 gm. orally (6)	1.5 gm. injected + 6 gm. orally (6)	1.5 gm. injected + 6 gm. orally[5] in unfractionated serum (6)	For each route of administration total dosages were divided into five equal portions given 2, 4, 6, 8 and 10 hours post-partum. Oral doses were given by stomach tube.
2	Negative control, physiological saline (3)	Control + 11 mg. tetracycline[3] per kg. liveweight daily for 5 days (3)	1.5 gm. immune globulins injected + 20 gm. orally (2)	Immune globulins + tetracycline (3)	Positive control, nursed 24 hours (6)		Immune globulins administered as in Experiment 1. Tetracycline administered intramuscularly.
3	Negative control, physiological saline (3)	10 gm. immune globulins injected (3)	Positive control, nursed 24 hours (4)				Immune globulins administered in eight intraperitoneal injections at 2-hour intervals beginning 2-hours post-partum
4	Negative control, physiological saline (4)	Immune globulins 15, 10, 5, 5 and 5 gm. orally on days 1 to 5 respectively (4)					Immune globulins were mixed with milk replacer and fed in nursing bottles as previously outlined.
5	Negative control, physiological saline (4)	Immune globulins (University herd) 15, 10, 5, 5 and 5 gm. orally on days 1-5 respectively (4)	Immune globulins as in lot 2 + 10 gm. porcine albumin on days 1 and 2 (3)	Immune globulins (pooled abattoir serum) as in lot 2 (4)	Reared on sow[4] (4)		Immune globulins and albumin fed as in Experiment 4.

[1] Number of pigs per lot shown in parentheses
[2] Indicated dosages per kg. liveweight in all experiments
[3] Achromycin, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.
[4] This lot not included in statistical comparisons
[5] The maximum volume which could be administered without severe stomach distension

TABLE 3.—SURVIVAL DATA AND SERUM PROTEIN DISTRIBUTION. *EXPERIMETN 1.*

Treatment	Per cent survival to: (weeks)			Component	Mean values of serum protein components (gm./100 ml.)								
					Age								
	1	2	12		birth	2 days	1 wk.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
1. Negative control	33	33	0	Albumin	0.43	0.48	0.81	1.33	2.20	2.70	2.07	2.20	
				Alpha$_2$ globulin	0.86	1.07	1.40	2.40	1.21	1.05	0.69	2.30	
				Beta globulin	0.39 *2.7*[1]	0.40	0.52 *4.8*	0.69 *5.1*	0.75 *13.0*	0.82 *26.5*	0.68 *38.0*	1.65	
				Gamma globulin	0.10	0.11	0.13	0.17	0.19	0.21	0.29	0.67	
				Total protein	1.78	2.06	2.86	4.59	4.35	4.78	3.73	6.82	
2. Immune globulins 1.5 gm. i.p.	33	33	17	Albumin	0.47	0.56	1.24	1.44	2.08	3.00	2.36	2.88	2.50
				Alpha$_2$ globulin	0.81	1.04	1.24	1.53	1.16	1.22	1.25	1.94	2.12
				Beta globulin	0.38 *2.7*	0.55	0.56 *4.5*	0.63 *5.6*	0.68 *13.0*	0.94 *25.5*	0.98 *37.5*	1.16	1.49 *58.0*
				Gamma globulin	0.10	0.38	0.29	0.22	0.24	0.27	0.46	0.90	1.20
				Total protein	1.76	2.59	3.34	3.82	4.16	5.43	5.05	6.88	7.31
3. Immune globulins 20 gm. oral	33	33	33	Albumin	0.32	0.56	0.94	1.14	2.26	2.78	2.99	2.88	3.18
				Alpha$_2$ globulin	0.78	1.23	1.26	1.64	0.88	0.99	1.08	1.25	1.38
				Beta globulin	0.46 *2.5*	1.09	0.87 *3.6*	0.82 *4.8*	0.67 *11.3*	*0.81 23.0*	0.90 *36.5*	1.04 *43.0*	1.10 *69.5*
				Gamma globulin	0.14	1.78	1.05	0.48	0.24	0.20	0.24	0.48	0.70
				Total protein	1.70	4.66	4.12	4.08	4.05	4.78	5.21	5.65	6.36
4. Immune globulins 1.5 gm. i.p. 20 gm. oral	67[2]	33	33	Albumin	0.44	0.50	0.88	1.50	2.23	2.50	2.14	3.02	3.06
				Alpha$_2$ globulin	0.97	1.05	1.86	1.70	0.84	1.54	1.40	1.20	1.20
				Beta globulin	0.40 *2.5*	0.86	0.92 *3.1*	0.84 *4.8*	0.52 *11.0*	0.96 *22.3*	1.10 *35.0*	0.98 *46.0*	1.04 *69.5*
				Gamma globulin	0.12	1.78	1.19	0.60	0.23	0.33	0.48	0.52	0.61
				Total protein	1.94	4.19	4.85	4.64	3.82	5.33	5.12	5.72	5.91
5. Immune globulins 1.5 gm. i.p. 6 gm. oral	0	0	0	Albumin	0.37	0.50							
				Alpha$_2$ globulin	1.00	1.22							
				Beta globulin	0.63 *2.3*	0.98							
				Gamma globulin	0.13	0.88							
				Total protein	2.13	3.58							
6. Immune globulins 1.5 gm. i.p. 6 gm. oral (serum)	67[2]	67[2]	67[3]	Albumin	0.38	1.66	1.73	1.18	2.43	2.59	2.52	2.34	2.48
				Alpha$_2$ globulin	0.94	1.37	1.70	1.53	1.19	1.28	1.66	1.79	1.68
				Beta globulin	0.51 *2.5*	0.90	0.92 *3.1*	0.71 *4.4*	0.78 *8.6*	0.89 *18.7*	1.25 *29.7*	1.34 *40.2*	1.22 *56.2*
				Gamma globulin	0.09	1.06	0.92	0.45	0.31	0.28	0.62	0.66	0.98
				Total protein	1.92	4.99	5.27	3.87	4.71	5.04	6.05	6.13	6.34

[1] Mean weight of surviving pigs on each bleeding date is given in italics.
[2] Percentage survival significantly higher than in Lot 5. ($P < 0.05$)
[3] Percentage survival significantly higher than in Lots 1, 2 and 5. ($P < 0.05$)

Experiment 5 was designed to obtain additional data on extended oral administration of immune globulins, to study the effects of adding porcine albumin to the globulin solution, and to compare immune globulins obtained from the University herd with those from pooled abattoir blood.

Experimental treatments, and details of methods of administration are shown in Table 2. In each experiment the volume of material administered was equalized for all treatments by appropriate dilutions with physiological saline.

RESULTS

Experiment 1 (Oral vs. parenteral globulin administration; fractionated globulins vs. serum)

Survival and serum protein composition data are summarized in Table 3. Survival was significantly better in the pigs receiving serum (Lot 6) than in the controls or those receiving globulins parenterally or orally at the lower level (Lots 1, 2 and 5).

Mortality was attributed to colibacillosis. Characteristic signs of this disease, which was responsible for practically all the mortality in these experiments, included severe yellowish-white diarrhoea, anorexia and emaciation. Post-mortem examination usually revealed a severe enteritis, and in some instances an extensive subcutaneous edema, as well as edema involving the lungs and other internal organs. The diagnosis of colibacillosis was based on the isolation of *E. coli* from cultures of heart blood, lung or liver.

Average weight gains ranged from 54 to 67 pounds for the 12-week experimental period but did not differ significantly between treatments. Growth was generally poor during the first 2 weeks, with marked improvement occurring thereafter.

Albumin levels increased slowly during the first week and more rapidly thereafter. In Lot 6 the increase was significantly greater at 2 days of age than in other lots, in which deficiencies remained very obvious during the first week.

The β-globulin level increased in all treated lots during the first 2 days. Increases in Lots 3, 4 and 6 were significantly greater than in Lot 1. Some decrease occurred in all except Lots 1 and 2 between 2 days and 2 weeks of age.

In all lots receiving globulins, marked increases in γ-globulin levels, proportional to the quantities given, were noted. The increase was significantly higher in Lots 3 and 4 than in Lots 1, 2 or 5, and significantly higher in Lot 6 than in Lots 1 or 2. The elevation in Lot 5 was, however, significantly higher than in Lot 1 only, suggesting that the immune globulins in serum were absorbed more efficiently than fractionated material. A steady decline in γ-globulin levels to minima at 4 to 6 weeks of age occurred in all treated lots. Similar declines were noted in subsequent experiments, and have been reported by others (16, 20).

TABLE 4.—SURVIVAL DATA AND SERUM PROTEIN DISTRIBUTION. *EXPERIMENT 2.*

Treatment	Per cent survival to: (weeks)			Component	Mean values of serum protein components (gm./100 ml.)								
					Age								
	1	2	12		Birth	2 days	1 wk.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
1. Negative control	33	33	33	Albumin	0.47	0.52	0.60	0.80	1.12	1.21	2.36	1.75	2.05
				Alpha$_2$ globulin	0.86	0.96	1.28	1.78	1.28	1.62	1.16	0.95	1.50
				Beta globulin	0.53 *2.9*[1]	0.33	0.53 *3.5*	1.00 *4.2*	2.11 *5.8*	1.21 *8.7*	1.33 *18.6*	2.23 *25.0*	1.21 *37.0*
				Gamma globulin	0.14	0.19	0.40	0.76	1.92	2.04	1.53	1.23	1.00
				Total protein	2.00	2.00	2.81	4.34	6.43	6.08	6.38	6.16	5.76
2. Control + achromycin	0	0	0	Albumin	0.47	0.47							
				Alpha$_2$ globulin	0.82	0.76							
				Beta globulin	0.42 *2.6*	0.56							
				Gamma globulin	0.09	0.13							
				Total protein	1.80	1.92							
3. Immune globulins 1.5 gm. i.p. 20 gm. oral	0	0	0	Albumin	0.23	0.73							
				Alpha$_2$ globulin	0.70	1.52							
				Beta globulin	0.43 *2.1*	0.96							
				Gamma globulin	0.10	2.10							
				Total protein	1.46	5.31							
4. Immune globulins + achromycin	67	0	0	Albumin	0.40	0.70	0.64						
				Alpha$_2$ globulin	0.88	1.27	1.71						
				Beta globulin	0.58 *2.2*	1.05	0.88 *2.3*						
				Gamma globulin	0.22	2.17	1.66						
				Total protein	2.08	5.19	4.89						
5. Nursed 24 hours	33	0	0	Albumin									
				Alpha$_2$ globulin									
				Beta globulin									
				Gamma globulin									
				Total protein									

[1] Mean weight of surviving pigs on each bleeding date is given in italics.

Experiment 2 (*Parenteral plus oral administration; effects of achromycin*)

The results of this experiment are given in Table 4. Treatment differences in percentage survival were not significant, all pigs except one control having died before reaching 2 weeks of age. This animal was apparently abnormal with respect to serum protein pattern. Characteristic signs of colibacillosis were noted prior to death, and pure culture of *E. coli* were isolated from the three pigs in which a positive diagnosis was possible. The antibiotic had no effect on survival.

Increases in β- and γ-globulin levels during the first 2 days were noted in Lots 3 and 4 as compared with Lots 1 and 2. The increase was significantly higher in the case of γ-globulin. In the surviving control pig, values for both β- and γ-globulin increased very rapidly between 2 days and 4 weeks of age. This was considered to be an abnormal occurrence, as no such increase was found in any other pig studied. It might be noted here, however, that Olsson (18) has reported marked variation in the age at which young pigs first begin to synthesize antibodies.

Experiment 3 (*Effects of high levels of globulins administered parenterally*)

Results are summarized in Table 5. None of the differences in percentage survival was significant. Deaths were attributed to *E. coli* infection in all except one pig.

The growth rate of the surviving pig in Lot 2 was substantially lower than that of the two suckled animals. The former gained 47 pounds between birth and 12 weeks, as compared to an average of 67 pounds for the latter.

No appreciable change in the level of any serum protein component occurred in Lot 1 during the first 2 days. In Lots 2 and 3 similar and highly significant increases in β- and especially γ-globulin were observed. A characteristic decline in γ-globulin to minima at 6 to 8 weeks of age was again found.

Experiment 4 (*Prolonged oral administration of globulins*)

Survival and serum protein composition data are summarized in Table 6. All treated pigs, and two of the four control animals survived the 12-week experimental period. The difference in percentage survival was not significant. The fact that no mortality and no obvious symptoms of illness occurred among the treated pigs was, however, taken to indicate beneficial effects from the immune globulins. The surviving controls had severe diarrhoea during the first week and remained unthrifty. Both these animals died shortly after removal to the piggery at 12 weeks of age.

The results of this experiment focused attention on the importance of birth weights in relation to survival in an artificial rearing program. This factor, which is discussed later, proved critical. All of the pigs in Experiment 4 weighed 2.7 pounds or more at birth, and all were exceptionally strong.

Treated pigs grew at a significantly greater rate than the controls. The average weight gain from birth to 12 weeks was 51 pounds for the controls as compared to 67 pounds for the treated animals.

TABLE 5.—SURVIVAL DATA AND SERUM PROTEIN DISTRIBUTION. *EXPERIMENT 3.*

Treatment	Per cent survival to: (weeks)			Component	Mean values of serum protein components (gm./100 ml.)								
					Age								
	1	2	12		Birth	2 days	1 wk.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
1. Negative control	33	0	0	Albumin	0.57	0.49	0.33						
				Alpha$_2$ globulin	0.73	1.10	1.02						
				Beta globulin	0.34 *2.8*[1]	0.40	0.23 *2.1*						
				Gamma globulin	0.11	0.12	0.10						
				Total protein	1.75	2.11	1.68						
2. Immune globulins 10 gm. i.p.	33	33	33	Albumin	0.35	0.59	0.57	1.00	2.53	2.51	2.89	2.24	2.17
				Alpha$_2$ globulin	0.80	1.02	1.66	1.52	0.96	0.84	1.00	2.03	2.19
				Beta globulin	0.39 *2.8*	1.28	1.10 *2.6*	0.90 *4.4*	0.59 *7.7*	0.51 *15.7*	1.00 *26.0*	0.78 *38.0*	1.24 *50.0*
				Gamma globulin	0.09	3.44	2.11	0.73	0.38	0.30	0.33	0.38	0.79
				Total protein	1.63	6.33	5.44	4.15	4.46	4.16	5.22	5.43	6.39
3. Nursed 24 hours	75	50	50	Albumin	0.44	1.13	1.30	1.26	2.12	2.58	3.16	3.16	3.18
				Alpha$_2$ globulin	0.88	1.62	1.30	1.66	0.92	0.84	0.94	0.88	0.97
				Beta globulin	0.40 *3.0*	1.47	1.45 *2.8*	0.86 *4.0*	0.70 *7.2*	0.88 *17.0*	0.74 *30.5*	0.96 *45.5*	0.90 *60.5*
				Gamma globulin	0.12	3.47	2.42	1.32	0.72	0.32	0.26	0.36	0.49
				Total protein	1.84	7.69	6.47	5.10	4.46	4.62	5.10	5.36	5.54

[1] Mean weight of surviving pigs on each bleeding date is given in italics.

TABLE 6.—SURVIVAL DATA AND SERUM PROTEIN DISTRIBUTION. *EXPERIMENT 4.*

Treatment	Per cent survival to: (weeks)			Component	Mean values of serum protein components (gm./100 ml.)								
					Age								
	1	2	12		Birth	2 days	1 wk.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.[1]	12 wk.
1. Negative control	50	50	50	Albumin	0.52	0.47	0.80	1.14	1.60	2.28	2.46	2.91	2.21
				Alpha$_2$globulin	1.02	0.99	1.58	1.10	1.02	1.50	1.24	1.30	1.34
				Beta globulin	0.58 *3.3*[1]	0.40	0.60 *3.3*	1.00 *4.5*	1.32 *7.9*	0.82 *14.6*	0.72 *24.5*	0.96 *43.0*	0.68 *54.5*
				Gamma globulin	0.18	0.11	0.18	0.14	0.20	0.34	0.30	0.38	0.34
				Totel protein	2.30	1.97	3.16	3.38	4.14	4.94	4.72	5.55	4.57
2. Immune globulins	100	100	100	Albumin	0.43	0.50	1.08	1.28	2.18	2.58	2.47	2.65	2.41
				Alpha$_2$ globulin	1.05	0.79	1.11	1.12	1.39	0.88	0.94	0.96	1.16
				Beta globulin	0.45 *3.0*	0.81	0.94 *3.7*	0.62 *4.7*	0.62 *10.3*	0.82 *19.8*	0.94 *33.3*	0.88 *50.8*	0.80 *70.0*
				Gamma globulin	0.11	0.81	0.69	0.54	0.35	0.24	0.25	0.32	0.42
				Total protein	2.04	2.91	3.82	3.56	4.54	4.52	4.60	4.81	4.79

[1] Mean weight of surviving pigs on each bleeding date is given in italics.

Levels of β- and γ-globulins were increased significantly during the first 2 days in lot 2. The increase was somewhat less than in previous experiments where larger doses had been given during the first 24 hours.

Experiment 5 (Prolonged oral administration of globulins; effects of adding albumin; effects of globulin source)

Results of Experiment 5 are summarized in Table 7. Porcine albumin (Lot 3) resulted in a significant improvement in survival over the control pigs and those receiving pooled (abattoir) immune globulins. Although mortality was eventually heavy in the lots receiving only immune globulins, it should be noted that no diarrhoea or other symptoms of illness occurred in any of the treated pigs during the first 6 days. This observation was in marked contrast to those in earlier experiments where immune globulins had been fed for 24 hours only. As in Experiment 4, no signs of colibacillosis (to which mortality was again attributed) appeared while the pigs were receiving the globulins. This was interpreted as a positive indication that a protective action was being afforded. Most of the mortality among treated pigs occurred on the ninth, tenth and eleventh days.

Albumin values were significantly higher in Lot 3 than in Lots 1, 2 or 4 during the first week. A deficiency of this component was evident in Lots 2 and 4 during this period.

The amount of β-globulin present at 2 days of age was not significantly affected by treatment. Unusually high birth values, which may have masked treatment effects, were noted in some pigs in Lots 3 and 4.

Increases in γ-globulin values between birth and 2 days of age reflected treatments, and were significantly greater in treated than in the control lot. The increase in Lot 3, furthermore, was significantly greater than in Lots 2 or 4, a finding which was somewhat analogous to that previously noted in Experiment 1, Lot 6.

Alpha$_2$ globulin levels did not appear to be associated with survival or growth rate in any of the experiments. Total protein values at 2 days of age generally reflected the amount of porcine proteins administered. Thereafter an increase, which could be accounted for mainly by albumin synthesis, was noted.

Representative electrophoretic patterns from certain of the experimental treatments are shown in Figure 1.

DISCUSSION

The relatively poor survival of pigs nursed for 24 hours, coupled with the fact that no symptoms of disease were evident during the 5-day period of oral administration of immune globulins, suggests that this method of administration was superior. It is presumed that the immune globulins were active as coproantibodies.

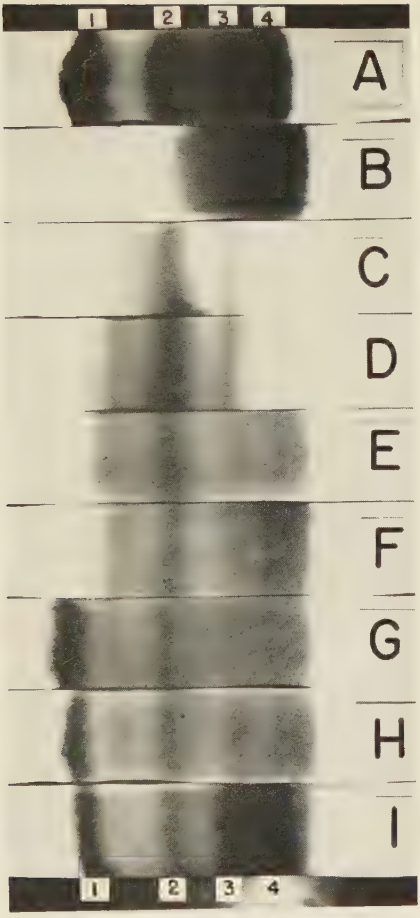

FIGURE 1. Representative electrophoretic patterns. A — mature porcine serum; B — immune globulins as administered; C + D — negative controls at birth and 2 days; E — immune globulins 1.5 gm. i.p.; F — immune globulins 20 gm. oral; G — immune globulins 1.5 gm. i.p., 6 gm. oral (serum); H - immune globulins + albumin; I — suckled pig. Patterns D to I inclusive are from 2-day-old pigs. No. 1, 2, 3 and 4 indicate albumin, α_2-globulin, β-globulin and γ-globulin respectively.

TABLE 7.—SURVIVAL DATA AND SERUM PROTEIN DISTRIBUTION. *EXPERIMENT 5.*

Treatment	Per cent survival to: (weeks)			Component	Mean values of serum protein components (gm./100 ml.) Age								
	1	2	12		Birth	2 days	1 wk.	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.
1. Negative control	50	0	0	Albumin	0.30	0.33	0.44						
				$Alpha_2$ globulin	0.64	0.69	1.36						
				Beta globulin	0.62 *2.3*[1]	0.56	0.72 *3.1*						
				Gamma globulin	0.08	0.19	0.13						
				Total protein	1.64	1.77	2.65						
2. Immune globulins (U. of S. serum)	100[2]	25	25	Albumin	0.64	0.53	0.87		2.48	2.40	2.09	2.33	2.26
				$Alpha_2$ globulin	1.23	1.04	1.22		1.08	1.14	1.41	1.55	1.03
				Beta globulin	0.46 *2.5*	0.74	0.92 *3.5*		0.75 *6.6*	0.66 *15.0*	0.74 *20.0*	1.10 *31.0*	1.97 *39.0*
				Gamma globulin	0.12	0.62	0.43		0.26	0.22	0.21	0.43	0.48
				Total protein	2.45	2.93	3.44		4.57	4.42	4.45	5.41	5.74
3. Immune globulins (U. of S. serum) + albumin	100[2]	67[3]	67[3]	Albumin	0.53	1.22	1.58	2.36	2.57	2.82	2.70	3.42	3.24
				$Alpha_2$ globulin	1.03	0.94	1.05	1.32	0.80	0.79	0.90	1.06	1.47
				Beta globulin	0.90 *2.8*	0.98	0.86 *3.9*	0.70 *4.7*	1.02 *7.0*	0.69 *17.5*	0.78 *33.0*	1.17 *57.5*	0.91 *74.5*
				Gamma globulin	0.12	0.99	0.69	0.54	0.28	0.23	0.25	0.44	0.50
				Total protein	2.58	4.13	4.18	4.92	4.67	4.53	4.63	6.09	6.12
4. Immune globulins (abattoir serum)	100[2]	0	0	Albumin	0.45	0.55	0.82						
				$Alpha_2$ globulin	0.86	0.97	0.90						
				Beta globulin	0.98 *2.2*	0.64	0.93 *3.2*						
				Gamma globulin	0.10	0.44	0.28						
				Total protein	2.39	2.60	2.93						
5. Reared on sow	—	—	—	Albumin		1.46	1.89	2.53	2.51	3.21	3.36	3.29	3.08
				$Alpha_2$ globulin		1.29	1.69	1.37	0.82	0.89	0.95	0.92	1.65
				Beta globulin		2.33	1.25	0.81	1.14	0.86	1.26	0.76	1.56
				Gamma globulin		2.69	1.52	0.56	0.34	0.32	0.36	0.50	0.80
				Total protein		7.77	6.35	5.27	4.81	5.28	5.93	5.47	7.09

[1] Mean weight of surviving pigs on each bleeding date is given in italics.
[2] Difference between Lot 1 and Lots 2, 3 and 4 approached significance ($P < 0.05$)
[3] Significantly higher than in Lots 1 and 4 ($P < 0.05$)

These indications of a need for a continuing supply of immune globulins in the lumen of the gut, and the inadequacy of parenteral administration, are not in agreement with the findings of Johnson *et al.* (13) and Lehrer *et al.* (14), who found that pigs nursing for 24 and 48 hours respectively could be successfully reared on synthetic diets. They are in contrast also to the findings of Pond *et al.* (19), who reported that intraperitoneal injection of γ-globulin and antibiotics during the first 24 hours provided adequate protection for colostrum-deprived pigs. Valid comparisons are not possible, however, between the various investigations because of variations in the degree of exposure to infection that may have existed. Much greater attention to sanitation appears to have been given by Pond *et al.* (19) than was the case in the experiments reported herein.

On the other hand, the findings of Speer (23) in which porcine γ-globulin gave a growth response in early-weaned pigs, as well as the established importance of coproantibodies in certain human diseases either originating in or confined to the intestinal tract, can be cited in support of the contention that the action of orally administered immune globulins as coproantibodies in these experiments was of great importance. It is possible, too, that the lower volumes administered during the first 24 hours in those experiments (4 and 5) where globulins were fed for 5 days may have, in lessening distension in the intestinal tract, reduced the susceptibility to infection.

The data suggest that albumin, supplied during the first 2 days of life either in serum or as a semi-purified fraction, produced a superior response. It is of interest that in pigs given only immune globulins, a marked deficiency of albumin was evident at 2 days of age. The effect of albumin was to create a serum protein pattern similar to that found in suckling pigs. The fact that the presence of albumin enhanced the absorption of the immune globulins in Experiment 1 (Lot 6) and Experiment 5 (Lot 3) raises the possibility that the albumin exerted an indirect protective action against enzymatic digestion of the globulins, thereby facilitating their absorption in the intact state.

The 100 per cent mortality in Lot 4, Experiment 5 was not regarded as conclusive evidence that immune globulins from slaughter house blood were ineffective. This material apparently afforded complete protection during the time it was being administered, and the results may have been complicated by the fact that the pigs used in Experiment 5 were generally smaller and less robust at birth than those in Experiment 4.

An analysis of survival as related to birth weight indicated the latter to be a significant factor, the critical birthweight being approximately 3 pounds. The precise reason for improved survival in heavier pigs was not apparent. It was assumed, however, that these animals were in a more advanced state of physiological development as far as disease resistance was concerned. It is possible too that they were affected to a lesser extent by the stress of an artificial diet. A highly significant correlation ($r = 0.318$; $P < 0.01$) between birth weight and level of albumin in the blood serum at birth was noted. This might be interpreted as indicating advanced development, since

synthesis of this component normally begins a few days after birth. There was no indication of any association between birth weight and levels of α_2, β- or γ-globulin at birth, nor was the ability to absorb immune globulins influenced by birth weight.

The generally poor growth of the pigs during the first 2 weeks can be attributed, at least in part, to suboptimum protein and energy intake. Preliminary experiments* had indicated, however, that the feeding of either a higher solids (15 per cent) milk replacer, or larger quantities of the milk replacer finally selected, seriously aggravated the ever-present scouring problem. Similar findings have been reported by Pond *et al.* (19). Frequent feedings of the amounts indicated for Experiments 4 and 5 proved most suitable.

Survival records were continued on the pigs following their removal to the piggery and contact with other pigs at 12 weeks of age. Seventeen treated pigs and three negative control animals were moved to the barn. Five of the treated animals and all the controls died before reaching maturity (200 pounds). The difference in mortality between treated and control animals was statistically significant. Six of the eight pigs died within 28 days of removal to the piggery, and mortality in all instances was attributed to Glasser's disease (infectious serositis). This disease had killed a number of normally-raised pigs in the weeks immediately preceding, so that the death of five of the treated pigs was considered of no particular significance. The significant difference in mortality between treated and control pigs was, however, a definite indication that the latter in some way lacked disease resistance. These animals had remained unthrifty throughout the experimental period. The difference could not be associated with any deficiency in serum protein components at 12 weeks of age.

Survival to maturity in treated pigs (pooling all treatments except positive control) weighing 3 pounds or more at birth was 71 per cent compared to 11 per cent for pigs weighing less than 3 pounds. Negative control pigs failed to survive in every instance.

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THE INFLUENCE OF DIETARY VITAMIN A ON THE WEIGHT INCREASE, FOOD UTILIZATION AND NITROGEN RETENTION OF THE RAT

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ABSTRACT

Two experiments designed to study the influence of dietary vitamin A on the weight gains, food consumption, food digestibility, energy digestibility, nitrogen digestibility and nitrogen retention of growing rats were conducted. Both experiments employed purified diets to which vitamin A was added in the form of an alcoholic solution. The first experiment involved levels of 0.0, 0.1, 1.0, 10.0, 100 and 1,000 I.U. vitamin A per gram of food. Each of the above-mentioned criteria was measured weekly for 3 consecutive weeks. No variations associated with dietary vitamin A were detected. The second experiment was similar to the first but in addition included vitamin A at 10,000 I.U./gram feed and was continued for 7 consecutive weeks. The highest level of vitamin A was toxic, the rats dying within 10 days, but did not appear to affect food utilization. No other major effects attributable to vitamin A were observed. It was apparent that the growing rat can tolerate a wide range of concentrations of vitamin A in the food.

INTRODUCTION

Vitamin A appears to exert profound effects upon the feed intake, weight gain and nutrient utilization of the young rat. Using pair-fed rats it was found that neither fat nor nitrogen absorption was impaired by vitamin A deficiency until the animals began to lose weight (13); however, nitrogen catabolism was increased by the deficiency as demonstrated by increased excretion of urinary nitrogen. Later it was reported (14) that two-fifths of the deficiency in body weight appeared to result from decreased food intake whereas three-fifths was caused by some other direct influence of vitamin A deficiency. Braman *et al.* (2) found that, although vitamin A-deficient rats made smaller weight gains and retained less nitrogen than did pair-fed controls, there were no differences in digestibility of the diets, nitrogen in the urine, heat production, gain of fat or gain in fat per gram of nitrogen gained, attributable to dietary vitamin A levels. Emerique (4) reported that the percentage of dietary nitrogen retained by young rats fed a vitamin A-deficient diet decreased as the severity of the deficiency increased; however, nitrogen absorption appeared to remain normal. It was observed (5) that vitamin A-deficient rats remained in positive nitrogen balance even when losing weight; this led to the hypothesis that continued storage of nitrogen which cannot be utilized brings about anorexia. Impairment of nitrogen retention by rats suffering from vitamin A deficiency was also reported by Brown and Morgan (3) but, unlike those of Emerique (5), some of their animals exhibited negative nitrogen balance. Mayer and Krehl (8) reported that, by increasing the level of protein in diets fed to vitamin A-deficient rats, efficiency of food and protein utilization, and survival time, were decreased while the severity of the deficiency symptoms was increased; further, it was found that one of the first signs of vitamin A deficiency was a depletion of the animal's vitamin C reserves.

Feed intake, weight gain and nutrient utilization may also be affected by an excessive intake of vitamin A. It has been reported (7) that the administration of vitamin A to rachitic rats, at a level of 1,000 I.U. per gram of body weight, caused the animals to enter negative nitrogen balance at a time when feed was still being consumed. Depression of the growth curve is a very sensitive measure of vitamin A toxicity according to Ames *et al.* (1) who found that, while 10,000 I.U./100 gram body weight/day allowed normal growth, 15,000 to 20,000 I.U. caused a marked depression. Similarly other workers (15) found that large doses of vitamin A caused rats to make slower gains, or to lose weight faster, than pair-fed controls. Neururkar and Sahasradbudhe (10) fed young rats 40,000 I.U. per day and noted a reduction in feed intake and a loss in body weight; it was further observed that nitrogen retention was depressed although nitrogen absorption was not markedly affected. The literature pertaining to the biochemistry and pathology of hypervitaminosis A has been reviewed by Nieman and Obbink (11); it is of interest to note that some workers have reported symptoms of scurvy associated with high levels of vitamin A intake.

The present report concerns two experiments with young rats designed to study the effects of varying concentrations of dietary vitamin A on weight change, feed intake, digestibility of feed, energy and nitrogen, and on nitrogen retention. There is little information available concerning the effect of vitamin A on these variables when fed at levels intermediate between the low and high levels known to result in deficiency and toxicity symptoms, respectively.

TABLE 1.—DIET FED TO BREEDING RATS FROM MATING TO WEANING

Ingredient	Per cent
White corn meal	68
Soybean oilmeal (50% protein)	10
Crude casein	15
Fishmeal (60% protein)	2.5
Dicalcium phosphate	2.0
Ground limestone	1.5
Iodized salt	0.5
Vitamin mix	0.5

Vitamin mix	Grams	Vitamin mix	Grams
Vitamin D_3 (16,550 I.C.U./gm.)	5.0	Inositol	0.3
Manganese sulphate	6.0	Biotin	0.01
Vitamin B_{12} (6 mg./lb.)	46.0	Thiamine HCl	0.1
Niacin	1.5	Folic acid	0.02
Riboflavin (1 gm./oz.)	6.5	Menadione	0.05
Calcium pantothenate	0.7	Choline chloride (25% mix)	40.0
Pyridoxine	0.3	Vitamin E (20,000 I.U./lb.)	14.0
Para-amino benzoic acid	0.3	White corn meal	106.0

MATERIALS AND METHODS

General

Adult female rats of the Sprague-Dawley strain were fed a chick starter diet (Table 1) from the time of mating until their young were 3 weeks of age. This diet had previously been successfully used to deplete the vitamin A reserves of chicks and was fed to the breeding rats in order to lower the vitamin A content of their milk. The young rats were weaned at 3 weeks of age at which time they were placed in individual cages where they received one of the basal diets described in Table 2. One week later the rats were weighed, allotted to treatments, placed in metabolism cages and fed the experimental diets.

Feed consumption and weight gain data were recorded weekly. The faeces and urine were collected and were assayed for gross energy and nitrogen; apparent digestible energy (A.D.E.), apparent digestible nitrogen (A.D.N.) and nitrogen retention values were determined each week with each individual rat. The management of the rats, the collection and assay procedures and the methods of calculation have been described previously (17).

Statistical analyses followed the procedures described by Snedecor (18) and Steel and Torrie (19). The variances apportioned to individual degrees of freedom were tested for significance by means of the studentized form of the extreme mean square test attributable to Nair (9).

Experiment 1

Weanling rats were fed diet 1 (Table 2) for 1 week prior to being put in the metabolism cages. Three male and three female rats were allotted to each of the six diet treatments which they received for 3 consecutive weeks. The diet treatments consisted of diet 1 supplemented with 0, 0.1, 1.0, 10, 100, and 1,000 I.U. of vitamin A per gram. The vitamin A was added to the diets in the form of alcoholic solutions of crystalline vitamin A acetate, 50 milliliters of ethyl alcohol containing the appropriate quantity of vitamin A being mixed with each kilogram of diet. The diets were stored in screw-cap glass jars in a refrigerator. Fresh diets were prepared at least once a week.

TABLE 2.—COMPOSITION OF THE BASAL DIETS

Ingredient	1	2
	per cent	per cent
Nitrogen source "A"[1]	11.4	—
Vitamin test casein	—	10.0
Purified cellulose[2]	10.0	15.0
Sucrose	68.6	65.0
Corn oil	5.0	5.0
Salts[3]	4.0	4.0
Vitamins[4,5]	1.0	1.0

[1]Vitamin test casein 587 gm., lactalbumen 688 gm., DL-methionine 5 gm., L-histidine HCl 15 gm., DL-threonine 10 gm., as used by Sibbald *et al.* 1956. (16).
[2]Alphacel "non-nutritive cellulose". Nutritional Biochemical Corp., Cleveland, Ohio.
[3]Jelinek *et al.* 1952. (6).
[4]Calciferol (120,000 I.U. vitamin D/gm.) 140 mg., thiamine HCl 50 mg., riboflavin 100 mg., pyridoxine HCl 50 mg., niacin 50 mg., Ca pantothenate 250 mg., folic acid 20 mg., biotin 2 mg., B_{12} 0.3 mg., α-tocopherol acetate 250 mg., menadione 20 mg., para-amino benzoic acid 3 gm., inositol 10 gm., dextrose 86 gm.
[5]A choline chloride solution (50 gm./100 ml.) was added at a rate of 4 ml./Kg. to supply 0.2 per cent choline chloride.

Experiment 2

This experiment was similar to the previous one. Basal diet 2 (Table 2) was used instead of diet 1. An extra treatment involving 10,000 I.U. of vitamin A per gram of feed was included and the number of rats per treatment was reduced to four males and one female. Vitamin A palmitate was used instead of vitamin A acetate. The experiment was extended to cover 7 consecutive weeks from the time the rats entered the metabolism cages. In all other respects the experiment was the same as the previous one.

RESULTS AND DISCUSSION

Experiment 1

One male rat receiving diet 3 (1.0 I.U. vitamin A per gram) and one female receiving diet 6 (1,000 I.U. per gram) died during the second week of the experiment. These animals exhibited no symptoms of either vitamin A deficiency or vitamin A toxicity; consequently, the data pertaining to these animals were eliminated from all calculations.

The mean responses of the rats to the six dietary vitamin A levels, as measured by several criteria, are presented in Table 3. It is apparent that the treatments exerted no major effects upon the several variables measured. Analyses of variance revealed sex differences in the weight gain, food con-

TABLE 3.—*EXPERIMENT 1.* MEAN RESPONSES OF RATS TO VARIOUS DIETARY VITAMIN A LEVELS

Criteria	Week of experiment	I.U. vitamin A per gram diet						
		0.0	0.1	1.0	10.0	100	1,000	C.V. %[1]
Weight gain (gm.)	1	32.8	31.4	31.9	34.3	33.9	32.8	17.4
	2	34.5	33.2	31.8	36.8	34.5	33.6	16.4
	3	30.6	29.2	30.7	30.9	29.4	29.5	15.9
Food consumption (gm.)	1	90	93	91	91	90	84	14.4
	2	117	117	116	124	115	111	12.5
	3	122	119	121	125	117	113	11.0
Food digestibility (%)	1	90	90	90	90	90	90	0.46
	2	90	90	90	90	90	89	0.63
	3	90	90	90	90	90	90	0.59
Energy digestibility (%)	1	91	90	90	91	90	90	0.41
	2	90	90	90	90	90	89	0.58
	3	91	91	91	91	90	90	0.59
Nitrogen digestibility (%)	1	92	92	92	93	93	92	1.72
	2	92	92	91	92	93	92	2.00
	3	90	91	90	91	92	91	2.87
Digestible nitrogen retained (%)	1	77	75	74	74	76	76	5.57
	2	73	70	71	70	70	73	6.51
	3	65	65	62	65	65	69	8.90

[1]C.V. coefficient of variation is $\frac{\sqrt{\text{Error mean square}}}{\text{Mean of experiment}} \times 100$

sumption and nitrogen retention data. Only the energy digestibility data, calculated to one significant decimal place, demonstrated a significant treatment effect. The linear ($P < 0.01$) decrease in energy digestibility which occurred as the vitamin A levels increased logarithmically was quite small and of doubtful importance.

The final column of Table 3 lists the coefficients of variation of each criterion by weeks. These data are of interest inasmuch as they indicate the variation of the responses of rats within a treatment. The calculation of these data was based on the mean squares remaining when the variation associated with replication and treatment was removed from the total variation. For the purpose of the analyses of variance sex was considered to be a treatment.

Initially it was planned that a number of other criteria should be employed to evaluate the influence of dietary vitamin A levels on the rat; however, as no major trends were observed in the data already considered it seemed impractical to extend the analyses of the data to include such measures as gain per unit of: food consumed, food digested, energy digested, nitrogen digested, nitrogen retained etc.

The results of this experiment demonstrate that the growing rat can tolerate a wide range of dietary vitamin A levels at least for a period of 3 weeks. No vitamin A deficiency symptoms were observed; hence it seemed probable that either the young rats had sufficient stores of vitamin A to last at least 3 weeks or that the basal diet contained some vitamin A or a precursor thereof. The basal diet, casein, lactalbumen, sucrose and cellulose when assayed by the method of Parrish and Smith (12) were found to contain neither vitamin A nor carotene.

Experiment 2

In view of the somewhat negative findings it was decided that the experiment should be repeated in a modified form. Nitrogen source "A" was replaced with vitamin test casein to remove the possibility that the lactalbumen might contribute vitamin A, or a precursor thereof, even though none could be demonstrated by chemical analysis. A level of 10,000 I.U. vitamin A per gram of diet was included in the design in an attempt to produce symptoms of hypervitaminosis A and, finally, the experiment was extended over 7 weeks to increase the opportunities for the rats to demonstrate chronic or acute deficiency or toxicity symptoms.

One male rat receiving diet treatment 5 (100 I.U. vitamin A per gram) died from unknown causes during the first week of the experiment. The data pertaining to this animal were eliminated from all calculations.

At no time during the 7 weeks of the experiment did any rat exhibit visible symptoms of vitamin A deficiency. The classical symptoms of vitamin A toxicity (kangaroo stance, fractured bones, hemorrhages, rough coat, reduced feed intake and loss of weight) were exhibited by all the rats receiving diet treatment 7 (10,000 I.U. vitamin A per gram). All rats of this group died within 10 days of the start of the experiment. The data pertaining to the individual animals of this group are presented in Table 4.

TABLE 4.—*EXPERIMENT 2.* DATA PERTAINING TO RATS FED A DIET CONTAINING 10,000 I.U. VITAMIN A PER GRAM

	Rat				
	A	B	C	D	E
Sex	♀	♂	♂	♂	♂
Initial weight (gm.)	41.5	48.0	46.0	43.0	39.0
Final weight (gm.)	34.2	38.0	36.2	35.7	33.7
Weight loss (gm.)	7.3	10.0	9.8	7.3	5.3
Food consumed (gm.)	29.2	20.6	31.0	45.8	29.9
Food digestibility (%)[1]	83.0	87.9	85.8	85.4	88.0
Energy digestibility (%)[1]	89.2	88.1	86.6	85.7	88.5
Nitrogen digestibility (%)[1]	81.1	90.9	87.9	90.1	92.4
Digestible nitrogen retained (%)[1]	22.5	22.5	48.7	50.1	54.4
Liver weight at death (gm.)	2.0	1.8	1.8	1.8	1.4
Survival time (days)	9	8	8	10	8

[1]Digestibility and retention data based on analyses of feces and urine collected during the first 7 days of the experiment

Although these rats lost weight and consumed less food than did the animals of the other experimental groups their ability to digest the diet, and the energy and nitrogen therein, was apparently unimpaired at least during the first week of exposure to the toxic diet. Nitrogen balance for the week as a whole was positive but low, suggesting that within a few days of the start of the experiment the animals entered negative nitrogen balance; this interpretation is supported by the weight change data. As the digestion of the dietary energy and nitrogen were normal it must be assumed that the negative nitrogen balance and loss of weight of rats receiving toxic levels of vitamin A are the result of reduced feed intake alone.

The data obtained from diet treatment groups 1 to 6 (0 to 1,000 I.U. vitamin A per gram of food) are presented as means in Table 5. Only two major trends are apparent, namely the increase in food consumption and the decrease in the amount of digestible nitrogen retained as the experiment progressed; however, such trends would be anticipated when animals are increasing in body weight. Although there appear to be differences between treatment groups no consistent trends associated with dietary vitamin A levels are apparent.

The data, obtained from the diet treatments listed in Table 5, calculated to one significant decimal place, were subjected to statistical analysis. The form of the analysis employed, together with the F-values obtained, is presented in Table 6. Although a number of significant differences were detected by the analysis their importance must be estimated in the light of the mean data of Table 5.

The first important difference detected by the analysis was the variation from week to week in the responses of the rats as measured by the criteria: weight gain, food consumption, food digestibility, energy digestibility, nitrogen digestibility and percentage digestible nitrogen retained. Apparently there was a linear relationship between the weight gains of the rats and the time on experiment; however, when the linear and quadratic terms were

TABLE 5.—*EXPERIMENT 2.* MEAN RESPONSES OF RATS TO VARIOUS DIETARY VITAMIN A LEVELS.

Criteria	Week of experiment	I. U. vitamin A per gram diet						
		0.0	0.1	1.0	10.0	100	1,000	Mean
Weight gain (gm.)	1	21.6	21.1	24.8	24.2	20.5	20.6	22.1
	2	18.4	16.9	18.1	18.5	15.4	13.1	16.7
	3	19.2	21.7	23.0	14.7	15.5	14.5	18.1
	4	27.3	26.3	29.3	24.9	20.2	21.1	24.8
	5	28.4	29.1	29.1	26.8	22.6	30.7	27.8
	6	23.0	22.5	18.6	24.1	19.5	26.2	22.3
	7	26.0	20.9	22.6	29.3	15.8	29.2	23.6
	Total	163.9	158.5	165.5	162.5	129.5	155.4	
Food consumed (gm.)	1	66	65	67	68	60	58	64
	2	75	73	78	76	67	62	72
	3	87	88	93	82	76	68	82
	4	101	99	108	88	87	81	94
	5	122	120	131	116	103	109	117
	6	130	135	130	134	113	127	128
	7	139	132	133	147	116	138	134
	Total	720	712	740	711	622	643	
Food digestibility(%)[1]	1	88	87	87	88	88	87	87
	3	87	86	87	86	86	87	86
	5	86	86	86	86	86	87	86
	7	85	86	86	86	85	85	85
Energy digestibility(%)[1]	1	88	87	88	88	88	88	88
	3	88	88	88	87	87	87	88
	5	87	87	87	87	87	87	87
	7	86	87	86	87	86	85	86
Nitrogen digestibility(%)[1]	1	92	92	92	94	93	93	93
	3	90	92	90	92	90	93	91
	5	92	92	92	93	91	94	92
	7	91	92	91	92	89	92	91
Digestible nitrogen retained (%)	1	81	78	80	75	75	79	78
	2	69	68	69	65	69	66	68
	3	64	63	68	61	66	65	65
	4	72	67	72	64	65	72	69
	5	68	66	66	65	64	70	66
	6	57	59	52	60	58	64	58
	7	58	54	50	58	53	58	55
	Mean	67	65	65	64	64	68	

[1]The food, energy and nitrogen digestibility data for weeks 2, 4 and 6 have been ommitted.

removed the residual mean square attributable to weeks was still large enough to be significant. This latter fact, together with the data of Table 5, suggest that linearity detected was fortuitous rather than real. Food consumption undoubtedly increased in a linear manner as the rats increased in body weight; this may be clearly seen in the mean data as well as in the results of the analysis. Although from a statistical standpoint both food and energy digestibility decreased in a linear manner as the experiment progressed the change was quite small. No trend associated with the time of the experiment could be detected in the nitrogen digestibility data; however, a signi-

TABLE 6.—SUMMARY OF THE F-VALUES RESULTING FROM THE ANALYSES OF THE DATA OF THE SECOND EXPERIMENT

Source of variation	D.F.	Weight change	Food consumption	Food digestibility	Energy digestibility	Nitrogen digestibility	Per cent digested nitrogen retained
Replications (R)	4	< 1.00	2.99*	1.65	1.20	< 1.00	5.28**
Weeks (W)	6	12.84**	152.13**	21.19**	22.92**	2.66*	60.23**
Linear (W_L)	1	21.67**	893.00**	87.58**	101.71**	< 1.00	283.92**
Quadratic (W_q)	1	< 1.00	< 1.00	1.87	< 1.00	< 1.00	< 1.00
R x W (Error A)	24						
Treatments (T)	5	4.25**	15.43**	2.52*	1.39	9.05**	5.32**
Linear (T_L)	1	5.90	46.56**	< 1.00	4.29	3.53	< 1.00
Quadratic (T_q)	1	< 1.00	8.52	7.62	< 1.00	3.25	21.31**
T x R	20	2.19**	6.99**	3.03**	3.24**	6.74**	2.58**
T x W	30	1.44	1.50	1.45	1.17	1.28	2.29**
T_L x W_L	1	2.44	< 1.00	< 1.00	3.52	2.61	6.96
T_q x W_L	1	7.33	2.04	7.02	9.46	1.10	3.76
T_L x W_q	1	4.38	7.12	< 1.00	< 1.00	< 1.00	< 1.00
T_q x W_q	1	< 1.00	1.86	5.28	3.27	1.70	< 1.00
T x R x W (Error B)	120						

**Indicates $P<0.01$
*$P<0.05$

ficant linear decrease may be noted in the percentage of digestible nitrogen retained.

Statistically significant treatment effects were detected for all criteria except energy digestibility. However, significant trends, associated with dietary vitamin A levels, were found only in the data pertaining to food consumption and nitrogen retention. The trend in the food consumption data was a linear decrease as dietary vitamin A levels increased logarithmically; however, as with the supposed trend in weight gains by weeks, the mean data of Table 5 suggest that, if such a linear relationship existed, it was not very strong. The statistical analysis suggests a quadratic relationship between the nitrogen retention data and the dietary vitamin A levels. This relationship is supported by the over-all mean treatment data of Table 5 but the differences between treatment means are quite small. If the means by weeks are examined the curvilinear effect is not readily detected. It is doubtful if either of these trends has much meaning as the differences between treatments are quite small and appear to be largely associated with differences in body weight.

The most interesting part of Table 6 is that concerned with the interactions between treatments and times. If the rats in any group suffered from chronic vitamin A deficiency or toxicity symptoms this section of the analysis should have revealed the fact. It will be seen that only one significant interaction was detected and that even this did not follow a specific linear or quadratic trend. It would appear that even after 7 weeks' exposure to the diet treatments there were no changes in the criteria studied which could be related to dietary vitamin A levels.

CONCLUSIONS

The findings of the experiments herein described demonstrated that growing albino rats can tolerate a wide range of dietary vitamin A levels for a period of 7 weeks without impairment of the body functions studied. A dietary level of 10,000 I.U. of vitamin A per gram was found to be toxic, causing death within 10 days.

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NOTE ON THE EFFECTS OF SELENIUM ON WOOL GROWTH AND BODY GAINS IN SHEEP[1]

Published results have shown that selenium will produce toxic symptoms when ingested in sufficient amounts by various classes of livestock (13). On the other hand, selenium has been found to be an important factor in the prevention of white muscle disease in lambs and calves (5, 8), exudative diathesis in chicks (11), and necrotic liver degeneration in rats and mice (4). McLean *et al.* (7) have reported increases in live-weight gains from 4.1 to 10.7 pounds due to selenium supplementation of lambs. Oldfield *et al.* (9) have obtained evidence that suggested an essential role for selenium in lamb growth. Because of the incidence of selenium poisoning and the widespread occurrence of selenious soil in Western Canada (1), a study on the effect of selenium on wool production and body weight gains in sheep was initiated at the Research Station, Lethbridge, Alberta, in 1956.

Two groups of five white-faced, range ewes were used in the experiment. In both years the ewes were bred and produced lambs normally. The control group received no selenium while the treatment group was given inorganic selenium (H_2SeO_3) orally. Concentrate pellets were fed at 2 pounds per head per day starting in November, 1956. For the treatment group the pellets contained 7.5 milligrams of selenium per pound. Both groups had access to good quality hay and water at all times. In September, 1957, the treatments were modified as follows to ensure proper dosage. Each ewe in both groups was given one-half pound of oats per day in addition to hay, and the selenium-supplemented ewes were given a daily drench containing 20 milligrams of inorganic selenium. Both groups were confined to pens and were group-fed throughout the experiment.

Data collected from both groups of ewes were: the amount of selenium in the blood and wool, the quantity and quality of wool produced by individual sheep, and body weights. Blood samples and skin biopsies were taken at the end of the experiment, wool samples after each shearing, and body weights bi-monthly. A spectrophotometric method (3) was used to analyse the blood and wool for selenium. For the clean wool determinations a composite sample of about 200 grams from the fleece of each sheep was used. The average fleece staple length was determind by measuring 10 locks from this sample. A random sample of 200 fibres was measured to obtain a mean fibre thickness for each fleece. A histological examination of skin samples from the two groups of sheep was made and the ratios of secondary to primary fibres (S/P ratios) were determined by standard techniques (2).

Blood analyses were not made the first year but during the second year of the experiment the selenium content of the blood from the treated group was double that of the control group (Table 1) and reached the level of intoxication (1.2-1.5 p.p.m.) observed by Rosenfeld and Beath (12). In the first year the selenium content of the wool from the treated group contained approximately 34 per cent more selenium than that from the control

[1]Contribution from the Animal Science and Soils Sections, respectively, Canada Agriculture Research Station, Lethbridge, Alberta.

group. A difference of 54 per cent was noted in the second year. Underwood (13) has stated that at intakes, which cause chronic poisoning, the selenium concentrations in certain tissues (liver, kidneys, and blood) continue to rise for long periods of time but tend to reach a maximum beyond which excretion appears to keep pace with absorption. This would appear to be the case in the present study. This also is in general agreement with the results of Leonard and Burns (6), who found that the blood selenium contents were 0.43, 1.16, and 1.98 p.p.m. while the wool selenium contents were 1.51, 2.26, and 1.44 p.p.m. from sheep grazed on low, medium, and high selenium areas, respectively. Rosenfeld and Beath (12) observed that the blood levels of selenium in sheep on a high protein ration increased from 0.69 to 2.51 p.p.m. after the administration of selenium for 116 days. Olson *et al.* (10) measured the selenium content of cattle hair and found that hair samples from animals on non-selenious areas ranged from less than 1 to 4 p.p.m. while those from selenious areas averaged over 10 p.p.m.

Treatment caused no differences in the wool production, staple length, and fibre thickness, based on data obtained from the 1957 wool clip. Results from the 1958 test showed an increase over the controls of 1.6 pounds per fleece ($p < .01$) of clean wool for the selenium-treated group. This increase was the result of greater staple length and a coarser fibre ($p < .05$). Leonard and Burns (6) found no differences in fibre length, fibre thickness, or fibre strength due to differences in the organic selenium content of the range. The average body weight of the selenium-drenched ewes was 13 pounds ($p < .01$) greater than the controls in 1957 and this difference persisted through 1958. This is in agreement with the results obtained by McLean *et al.* (7) and Oldfield *et. al.* (9).

The results of the histological examination of skin sections from selenium-treated and control ewes are given in Table 1. These results show that increasing the selenium content of the blood from 0.74 p.p.m. in the controls to 1.44 p.p.m. in the treated group had no effect on the S/P ratio or the number of primary follicles.

TABLE 1. — THE EFFECTS OF SELENIUM INTAKE ON FLEECE WEIGHTS, WOOL CHARACTERS, AND BODY WEIGHTS AND THE SELENIUM CONTENTS OF BLOOD AND WOOL

Group	1957		1958	
	Selenium-treated	Control	Selenium-treated	Control
Selenium in blood (p.p.m.)	–	–	1.44	0.74
Selenium in wool (p.p.m.)	5.12	3.82	5.30	3.44
Greasy fleece weight (lb.)	6.4	6.2	10.7[1]	8.1
Clean fleece weight (lb.)	3.9	3.8	5.9[1]	4.3
Staple length (mm.)	83	84	94	90
Fibre thickness (microns)	24	24	26[2]	22
Body weight (lb.)	131[1]	118	161[2]	149
S/P ratio	–	–	10.2	10.7

[1]Significantly greater than the control ($p < .01$)
[2]Significantly greater than the control ($p < .05$)

These tests show that the administration of sub-toxic levels of selenium produced an increase in clean fleece weight, fibre thickness, and body weight and suggest that selenium may be an essential nutrient for wool and body growth in sheep.

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NOTE ON THE VALUE OF FIRE- AND SMOKE-DAMAGED WHEAT FOR GROWING TURKEYS

During July, 1961, a disastrous fire at Wilcox, Saskatchewan, destroyed a number of elevators and damaged over 200,000 bushels of wheat. Inquiries from turkey growers as to its possible use in turkey rations led to the investigations reported in this paper.

Whiting (1) and Whiting and Bezeau (2) indicated that smoke-damaged grains were generally less palatable to livestock than ordinary grains and that it often required as long as 2 weeks for them to become accustomed to the odour and taste. They reported also that severely burned wheat was 18 per cent lower in digestibility than sound wheat, when fed to pigs, but that less severely damaged wheat was only 4 per cent less digestible.

The wheat recovered from the Wilcox fire was divided into three classes during salvage operations:

(a) *Wheat which was only slightly discolored.* It contained a small percentage of charred kernels, a moderate amount of charcoal in small pieces, and possessed a somewhat disagreeable odour.

(b) *Wheat showing considerable discoloration.* Practically all kernels showed some degree of darkening from smoke and adhering charcoal. The sample contained a higher percentage of charred kernels (21 per cent), a greater quantity of charcoal, and a very objectionable odour.

(c) *Wheat which could not be salvaged.*

A sample of wheat from class (b) was taken directly from the salvage piles. It was immediately sieved through a 5-mesh screen to remove the bulk of the charcoal and the nails. One per cent by weight was removed. It was then incorporated into a whole grain, pelleted concentrate turkey growing ration, of which it made up 67 per cent. The only other grain used in the ration was 23 per cent whole oats. Sixty-seven per cent of undamaged spring wheat was incorporated into an otherwise similar control ration.

The two rations were fed to 17-week-old turkey females, with each ration being replicated four times and using 10 birds per replicate. Feed consumption data were recorded at 1- to 4-day intervals, and the birds were weighed at the beginning and conclusion of the 3-week experimental period.

RESULTS

The taste and odour of the damaged wheat had no apparent effect on feed intake. During the first 24 hours the control groups averaged 0.317 pounds of feed per bird, while those on the damaged wheat averaged 0.325 pounds. Feed consumption at the end of the fourth day was identical at 0.389 pounds per bird per day.

Analysis of variance of the weekly feed consumption data showed no difference between treatments ($P = 0.05$). Total feed consumption per bird for the 21-day period amounted to 8.86 pounds for those on the control ration, and 9.04 pounds for those on the damaged wheat ration. There was no evidence that the birds tended to select out the undamaged kernels.

FIGURE 1. This photo shows the proportion of charred kerne's in Class "B" wheat. All of the charcoal was removed from this sample.

The gain data for the 21-day period indicated no difference between treatments ($P = 0.05$). The average gain per bird was 1.21 pounds on the control treatment and 1.15 pounds for those on the damaged wheat.

It is concluded that smoke- and fire-damaged wheat which resembles class (b) as discussed above is entirely satisfactory feed for growing turkeys.

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NOTE ON GRASS SILAGE IN RATIONS FOR WINTERING BEEF CALVES[1]

In Eastern Ontario a common practice is to winter beef calves on silage, hay and a small amount of a simple grain mixture. Corn silage, characterized by its comparatively low digestible crude protein (DCP) and high total digestible nutrients (TDN), is being replaced on many farms by grass silage which contains more than twice as much DCP but less TDN (Table 1). The question arises: Does the extra protein make grass silage superior to corn silage for wintering beef calves?

This report summarizes two experiments, each in two consecutive years, showing the effect of (a) grass silage vs. corn silage for wintering beef calves, disregarding the protein-energy ratio of the rations; (b) a simple vs. a complex concentrate to supplement a grass silage and hay ration for beef calves. In both experiments fall-weaned Shorthorn calves were used, divided equally according to sire, sex and weight into two lots. They were group-fed and maintained under loose housing conditions with free access to water and a mineral mixture of equal parts by weight of steamed bone meal and iodized salt.

Results given in Table 2 (Experiment I, Groups 1 and 2) show that there was no difference in the average daily gain of animals fed grass silage (timothy, red clover, alsike and alfalfa) or corn silage along with hay and the same grain mixture. DCP consumed by Group 1 exceeded that required for gain in body weight but the TDN consumed was the same for both groups. The results indicate that the protein required for the given level of energy was met by the corn silage ration. Energy may have been the limiting factor for not obtaining a greater gain from the grass silage ration containing a higher proportion of DCP.

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TABLE 1.—PER CENT DM, DCP AND TDN OF ROUGHAGES USED IN EXPERIMENTS I AND II (AS FED BASIS)

	DM			DCP			TDN		
	Grass sil.	Corn sil.	Hay	Grass sil.	Corn sil.	Hay	Grass sil.	Corn sil.	Hay
Experiment I									
Trial 1	23.1	19.7	87.2	1.89	.75	6.96	11.30	12.41	48.13
Trial 2	21.2	20.9	89.0	1.67	.70	5.52	11.48	13.83	48.75
Experiment II									
Trial 1	22.8		91.5	1.69		6.76	11.81		50.56
Trial 2	22.2		89.2	1.80		5.11	11.89		49.44

Note: Calculations of DM, DCP and TDN were based on actual chemical analyses of the feeds, but the digestibility coefficients used were for similiar feeds as listed in Morrison "Feeds and Feeding", 21st ed.

TABLE 2.—TWO-YEAR AVERAGE GAIN, FEED CONSUMPTION AND FEED EFFICIENCY OF FALL-WEANED SHORTHORN CALVES

Experiment No.		I		II	
Group		1	2	3	4
Rations		Hay, grass silage, grain	Hay, corn silage, grain	Hay, grass sil., simple concentrate	Hay, grass sil., complex concentrate
Animals per group	(no.)	33	33	36	37
Av. days on feed	"	116	116	128	128
Av. initial wt.	(lb.)	428.7	430.9	457.5	460.6
Av. daily gain	"	.79	.80	.76	.76
Av. feed/head/day					
Hay	(lb.)	3.99	3.99	4.50	4.60
Grain	"	1.86[1]	1.86[1]	2.00[2]	2.20[3]
Silage	"	18.69	18.25	19.71	19.70
DM	"	9.3	8.8	10.2	10.5
TDN	"	5.2	5.5	6.1	6.1
DCP	"	.83	.62	.82	.79
Av. feed/lb. gain					
DM	(lb.)	11.8	11.1	13.5	13.8
TDN	"	6.6	6.9	8.0	8.0
DCP	"	1.1	.78	1.1	1.04

[1]Oats and bran 3:1
[2]Oats and barley 3:2
[3]3oats: 2 barley: 2 beet pulp: 2 hydrated alfalfa meal: 1 cane molasses

Results obtained in Experiment II, Groups 3 and 4, indicate that there was no advantage in feeding a complex grain mixture along with grass silage and hay as compared to a simple mixture as both rations produced similar gain. It is interesting to note in Experiment II the daily TDN consumption was 1 pound higher than it was for the calves which were fed grass silage in Experiment I, yet no greater gains were obtained.

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Erratum:

In the paper by Peters *et al.* "Genetic trends in performance of the Romnelet sheep during the period of breed development" published in Vol. 41, No. 1 (June, 1961), the second sentence of the last paragraph in "Materials and Methods" (page 128) should read:

"In that year, the F1 lambs were out of 6-year-old ewes and the F2 lambs were out of 2-year-old ewes."